(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: USE OF ANTIBODIES TO THE GAMMA 2 CHAIN OF LALLMININ 5 TO INHIBIT TUMOR GROWTH AND METASTASIS

(57) Abstract: The present invention provides a methods and compositions for inhibiting tumor growth and/or metastasis involving the administering to a subject with a laminin 5-secreting tumor of an amount effective to inhibit tumor growth and/or metastasis of an antibody that binds to one or more epitopes of the laminin 5 γ2 chain.

Use of antibodies to the gamma 2 chain of laminin 5 to inhibit tumor growth and metastasis

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CROSS REFERENCE

This application claims priority to U.S. Provisional Patent Application 60/422,009 filed October 29, 2002.

10   **BACKGROUND OF THE INVENTION**

Laminins are basement membrane glycoproteins with diverse biological functions including cell adhesion, proliferation, migration and differentiation. Thus far, 11 genetically distinct chains forming at least 12 laminin isoforms have been characterized. Every member of this growing protein family has a heterotrimeric chain composition of α, β, and γ chains that are

15   formed through an intracellular self-assembly mechanism.

Laminin-5 is a specific component of epithelial basement membranes with the chain composition $\alpha3\beta3\gamma2$ (Kallunki, et al., J. Cell Biol. 119: 679-93, 1992). The $\gamma2$ chain has a mass of $\approx$ 130 kd and is thus smaller than the "classical" $\approx$ 200 kd $\beta1$ and $\gamma1$ light chains of laminin 1. Expression of laminin 5 chains is often up-regulated in epithelial cancers, such as squamous cell

20   carcinomas and gastric carcinomas, but not in mesenchymally derived cancers (Larjava, et al., J. Clin. Invest. 92: 1425-35, 1993) (Pyke, et al., Am. J. Pathol. 145: 782-91, 1994) (Pyke, et al., Cancer Res. 55: 4132-9, 1995) (Tani, et al., Am. J. Pathol. 149: 781-93, 1996) (Orian-Rousseau, et al., J. Cell. Sci. 111: 19932004, 1998) (Sordat, et al., J. Pathol. 185: 44-52, 1998). However, down-regulation has been reported in epithelial prostate and breast carcinomas (Hao, J., Yang,

25   Am. J. Pathol. 149: 1341-9, 1996) (Martin; et al., Mol. Med. 4: 602-613, 1998). In colon adenocarcinomas, both gene and protein expression of the $\gamma2$ chain seem to be a characteristic of cancer cells with a budding phenotype (Larjava, et al., J. Clin. Invest. 92: 1425-35, 1993) (Pyke, et al., Am. J. Pathol. 145: 782-91, 1994) (Pyke, et al., Cancer Res. 55: 4132-9, 1995). Tumor cell budding in colorectal carcinoma has also been associated with the presence of intracellular

30   laminin-5 (Sordat, et al., J. Pathol. 185: 44-52, 1998).

The γ2 chain of laminin-5 has also been shown to be strongly expressed in malignant cells located at the invasion front of several human carcinomas, as determined by *in situ* hybridization and immunohistochemical staining (Pyke, C., Romer, J., Kallunki, P., Lund, L.R., Ralfkiaer, E., Dano, K. & Tryggvason, K. (1994) Am. J. Pathol. 145: 782-791; Pyke, C., Salo, S.,

5   Ralfkiaer, E., Romer, J., Dano, K. & Tryggvason, K. (1995) Cancer Res. 55: 4132-4139). However, no studies have shown that antibodies to the γ2 chain of laminin 5 can be used to inhibit tumor cell growth.

SUMMARY OF THE INVENTION

10   The present invention provides antibodies, compositions and methods for inhibiting tumor growth and/or metastasis. In one aspect, the present invention provides antibodies that bind to one or more epitopes of domain III of the human laminin 5 γ2 chain (**SEQ ID NOS: 2 and 4**).

In another aspect, the present invention provides a method for inhibiting tumor growth

15   and/or metastasis comprising administering to a subject with a laminin 5-secreting tumor an amount effective to inhibit tumor growth and/or metastasis of an antibody that binds to one or more epitopes of the laminin 5 γ2 chain. In one embodiment, the antibody binds to one or more epitopes of domain III of the laminin 5 γ2 chain.

In a further aspect, the present invention provides a pharmaceutical composition

20   comprising an antibody that binds to the laminin 5 γ2 chain and one or more further anti-tumor agents. In various embodiments of this aspect, the antibody is selective for one or more epitopes in domain III of the laminin 5 γ2 chain, and/or the further anti-tumor agent is a chemotherapeutic.

25   **BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURE 1 shows efficiency of human laminin-5 and recombinant human laminin γ2 chain for attachment of HaCat keratinocytes and KLN205 squamous carcinoma cells *in vitro*. The attachment efficiency was compared with the efficiency with which the cells bound to laminin-1. Substrate concentrations (10 μg/ml) providing maximum attachment to laminin-1 and

30   laminin-5 were used. The results are presented as means +/- SD calculated from at least four duplicate series; the values for laminin-1 were given the arbitrary value of 100%.

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FIGURES 2A-B show the effects of polyclonal γ2 chain antibodies on the migration of KLN205 squamous carcinoma cells in Boyden and Transwell chamber assays of migration.

FIGURE 3 shows tumor growth inhibition using Mab 5D5 and CPT-11 on day 31 in the HT29-e28 cell line.

5   FIGURES 4A-E show tumor growth curves for individual mice in the HT29-e28 study.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the present invention provides a method for inhibiting tumor growth and/or

10   metastasis comprising administering to a subject with a laminin 5-secreting tumor an amount effective to inhibit tumor growth and/or metastasis of an antibody that binds to one or more epitopes of the laminin 5 γ2 chain. In a preferred embodiment, the subject is a mammal; in a more preferred embodiment, the subject is human.

As used herein, the term "inhibiting tumor growth" means to reduce the amount of tumor

15   growth that would occur in the absence of treatment, and includes decrease in tumor size and/or decrease in the rate of tumor growth.

As used herein, the term "inhibiting tumor metastasis" means to reduce the amount of tumor metastasis that would occur in the absence of treatment, and includes decrease in the number and/or size of metastases.

20   As used herein, the term "laminin-5 secreting tumor" means a tumor that expresses detectable amounts of laminin 5. Such tumors include, but are not limited to, carcinomas. Such carcinomas include, but are not limited to squamous cell carcinomas (including but not limited to squamous cell carcinoma of skin, cervix, and vulva), gastric carcinomas, colon adenocarcinomas, colorectal carcinomas, and cervical carcinomas.

25   As used herein, the term "laminin 5 γ2 chain" preferably refers to the human laminin 5 γ2 chain, with protein sequences comprising the amino acid sequence of **SEQ ID NO:2** or **SEQ ID NO:4**, and derivatives thereof.

As used herein, the term "epitope" refers to a specific site within the protein that is bound by the antibody, which includes both linear and non-linear epitopes.

30   In a preferred embodiment, the antibody binds to one or more epitopes of domain III of the laminin 5 γ2 chain. As used herein, the term "domain III of the laminin 5 γ2 chain" refers to

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a 177 amino acid region of **SEQ ID NO:2** between residues 391 and 567 (Kallunki et al., J. Cell Biol. 119:679-693 (1992)), which is presented herein as **SEQ ID NO:8**. In a further preferred embodiment, the antibody binds to one or more epitopes within domain III that are contained within the amino acid sequence of **SEQ ID NO:6** and does not bind to epitopes within domain

5   III that are within the amino acid sequence of **SEQ ID NOS: 9** and **10**.

The antibody can be a polyclonal antibody or a monoclonal antibody, but preferably is a monoclonal antibody. For use in humans, humanized monoclonal antibodies are especially preferred.

In a further embodiment, the methods of the invention further comprises treating the

10   subject with chemotherapy and/or radiation therapy, whereby the use of the antibody permits a reduction in the chemotherapy and/or radiation dosage necessary to inhibit tumor growth and/or metastasis. Any reduction in chemotherapeutic or radiation dosage benefits the patient by resulting in fewer and decreased side effects relative to standard chemotherapy and/or radiation therapy treatment.

15   In this embodiment, the antibody may be administered prior to, at the time of, or shortly after a given round of treatment with chemotherapeutic and/or radiation therapy. In a preferred embodiment, the antibody is administered prior to or simultaneously with a given round of chemotherapy and/or radiation therapy. In a most preferred embodiment, the antibody is administered prior to or simultaneously with each round of chemotherapy and/or radiation therapy.

20   The exact timing of antibody administration will be determined by an attending physician based on a number of factors, but the antibody is generally administered between 24 hours before a given round of chemotherapy and/or radiation therapy and simultaneously with a given round of chemotherapy and/or radiation therapy.

The methods of the invention are appropriate for use with chemotherapy using one or more

25   cytotoxic agent (ie: chemotherapeutic), including, but not limited to, cyclophosphamide, taxol, 5-fluorouracil, adriamycin, cisplatinum, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carbaplatinum, and vincristine. The cytotoxic agent can also be an antiviral compound which is capable of destroying proliferating cells. For a general discussion of cytotoxic agents used in chemotherapy, see Sathe, M. et al., Cancer Chemotherapeutic Agents: Handbook of

30   Clinical Data (1978), hereby incorporated by reference.

The methods of the invention are also particularly suitable for those patients in need of repeated or high doses of chemotherapy and/or radiation therapy.

In practicing the invention, the amount or dosage range of antibody employed is one that effectively inhibits tumor growth and/or metastasis. The actual dosage range is based on a variety

5   of factors, including the age, weight, sex, medical condition of the individual, the severity of the condition, and the route of administration. An inhibiting amount of antibody that can be employed ranges generally between 0.01 µg/kg body weight and 15 mg/kg body weight, preferably ranging between 0.05 µg/kg and 10 mg/kg body weight, more preferably between 1 µg /kg and 10 mg/kg body weight, and even more preferably between about 10 µg /kg and 5 mg/kg body weight.

10   The antibody may be administered by any suitable route, but is preferably administered parenterally in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term "parenteral" as used herein includes, subcutaneous, intravenous, intraarterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. In preferred embodiments, antibody is

15   administered intravenously or subcutaneously.

The antibody may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (e.g., solutions, suspensions, or emulsions). Antibody may be applied in a variety of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the antibody, and are not harmful for the proposed

20   application.

The antibody may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

For administration, the antibody is ordinarily combined with one or more adjuvants

25   appropriate for the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidine, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the antibody may be dissolved in

30   saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers.

Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

5   In another aspect, the present invention provides isolated antibodies that bind to one or more epitopes of domain III of the laminin 5 γ2 chain, hybridoma cells that produce isolated monoclonal antibodies, and pharmaceutical compositions comprising such monoclonals. In a further preferred embodiment, the isolated antibody binds to one or more epitopes within the amino acid sequence of **SEQ ID NO:6** and does not bind to epitopes within the amino acid sequence of **SEQ ID NOS: 9** and **10**. The isolated antibody can be a polyclonal antibody or a

10   monoclonal antibody, but preferably is a monoclonal antibody. In a further embodiment, the isolated antibodies are humanized. In a further embodiment, the isolated antibody is prepared as a pharmaceutical composition, combined with one or more appropriate pharmaceutical carriers, as described above.

       These isolated antibodies are useful in all of the methods of the invention, as well as in

15   diagnostic use for detecting the presence of invasive cells in a tissue sample. In a preferred embodiment, diagnostic use of the isolated antibodies of the invention comprises contacting a tumor tissue with one or more isolated antibodies to form an immunocomplex, and detecting formation of the immunocomplex, wherein the formation of the immunocomplex correlates with the presence of invasive cells in the tissue. The contacting can be performed *in vivo*, using

20   labeled isolated antibodies and standard imaging techniques, or can be performed *in vitro* on tissue samples.

       In a preferred embodiment, the tissue is a tumor tissue. In a further preferred embodiment, the tumor tissue is a laminin 5 secreting tumor tissue. More preferably, the tumor tissue is a carcinoma, including but are not limited to squamous cell carcinomas (including but

25   not limited to squamous cell carcinoma of skin, cervix, and vulva), gastric carcinomas, colon adenocarcinomas, colorectal carcinomas, and cervical carcinomas.

       In a further preferred embodiment of this aspect of the invention, the isolated monoclonal antibodies are of the IgG isotype. In a further preferred embodiment, the isolated monoclonal antibodies are selected from the group consisting of those designated herein as 4G1, 5D5 and

30   6C12, and the hybridomas expressing these monoclonals, which are deposited with the American Type Tissue Collection as ATCC accession numbers ----, ----, and ----. A more detailed

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description of the production of these particular hybridomas and monoclonal antibodies, and their use, is provided below.

The additional components of pharmaceutical compositions comprising one or more of these isolated antibodies are as described above.

5   Antibodies can be made by well-known methods, such as described in Harlow and Lane, Antibodies; A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1988). In one example, pre-immune serum is collected prior to the first immunization. A peptide portion of the amino acid sequence of a laminin 5 γ2 chain polypeptide, together with an appropriate adjuvant, is injected into an animal in an amount and at intervals sufficient to elicit

10   an immune response. Animals are bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. At about 7 days after each booster immunization, or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20° C. Polyclonal antibodies against the laminin 5 γ2 chain polypeptides can then be purified directly by

15   passing serum collected from the animal through a column to which non-antigen-related proteins prepared from the same expression system without the laminin 5 γ2 chain polypeptides bound.

Monoclonal antibodies can be produced by obtaining spleen cells from the animal. (See Kohler and Milstein, Nature 256, 495-497 (1975)). In one example, monoclonal antibodies (mAb) of interest are prepared by immunizing inbred mice with a laminin 5 γ2 chain polypeptide,

20   or portion thereof. The mice are immunized by the IP or SC route in an amount and at intervals sufficient to elicit an immune response. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of by the intravenous (IV) route. Lymphocytes from antibody positive mice are obtained by removing spleens from immunized mice by standard procedures known in the art.

25   Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner under conditions that allow formation of stable hybridomas. The antibody producing cells and fusion partner cells are fused in polyethylene glycol at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known

30   in the art. Supernatant fluids are collected from growth positive wells and are screened for antibody production by an immunoassay such as solid phase immunoradioassay. Hybridoma

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cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, Soft Agar Techniques, in Tissue Culture Methods and Applications, Kruse and Paterson, Eds., Academic Press, 1973.

5   To generate such an antibody response, a laminin 5 γ2 chain polypeptide or portion thereof is typically formulated with a pharmaceutically acceptable carrier for parenteral administration. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing Corynebacterium parvum and tRNA. The formulation of such compositions, including the concentration of the polypeptide and the selection of the vehicle and other components, is within the knowledge of those of skill

10   of the art.

        The term antibody as used herein is intended to include antibody fragments thereof which are selectively reactive with the laminin 5 γ2 chain polypeptides. Antibodies can be fragmented using conventional techniques, and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')₂ fragments can be generated by

15   treating antibody with pepsin. The resulting F(ab')₂ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

        In another aspect, the present invention provides pharmaceutical compositions comprising an antibody that binds to the laminin 5 γ2 chain and one or more further anti-tumor agent. In a preferred embodiment of this aspect of the invention, the antibody binds to one or

20   more epitopes in domain III of the laminin 5 γ2 chain, as described above. In a further preferred embodiment, the isolated antibody binds to one or more epitopes within the amino acid sequence of **SEQ ID NO:6** and does not bind to epitopes within the amino acid sequence of **SEQ ID NOS: 9 and 10**. The antibody can be a polyclonal antibody or a monoclonal antibody, but preferably is a monoclonal antibody. In a further preferred embodiment of this aspect of the

25   invention, the further anti-tumor agent is a chemotherapeutic agent, such as one or more of those described above. The components of the pharmaceutical composition may be pre-mixed together or may be combined at any time prior to administration to a patient in need thereof.

        The examples below are meant by way of illustration, and are not meant to be limiting as

30   to the scope of the instant disclosure.

EXAMPLE 1

The following example demonstrates the effect of laminin-5, including the γ2 chain of laminin-5, on cell adhesion and cell migration.

5   _Materials and Methods_

Cells and Cell Culture

A mouse squamous cell carcinoma cell line, KLN205 (cat. no. ATCC CRL-1453), was obtained from American Type Culture Collection (Rockville, MD). The cells were maintained as monolayer cultures in Eagle's minimum essential medium (MEM) containing non-essential

10   amino acids and Earle's BSS supplemented with 10% fetal calf serum (FCS). The HaCat human keratinocyte cell line was a kind gift from Dr. Fuzenig (Heidelberg, Germany). The HaCat cells were cultured in Dulbecco's MEM supplemented with 10% FCS. However, when the cells were cultured for the production of laminin-5, the medium was replaced by serum-free medium.

15   _Preparation of Proteins_

Mouse EHS laminin (laminin-1) was obtained from GIBCO BRL. Fibronectin was purified from FCS using a gelatin-Sepharose 4B column (Sigma) as described in Vuento, M. & Vaheri, A. (1979) Biochem. J. 183: 331-337.34 and Gillies, R. J., Didier, N. & Denton, M. (1986) Anal. Biochem. 159: 109-113. Human laminin-5 was immunoaffinity purified from the

20   media of HaCat cells cultured for three days in the absence of serum. Briefly, the medium was first passed through a 5 ml gelatin-Sepharose column (Sigma, St. Louis, MO) to ensure the complete absence of fibronectin from the protein preparation, after which the medium was passed through a 10 ml anti-laminin γ2-Sepharose affinity column in order to bind laminin-5 molecules. Both columns were equilibrated in phosphate-buffered saline. The anti-laminin γ2-

25   Sepharose affinity column was prepared by coupling a Protein A-purified anti-γ2 IgG (8 mg/ml) to 10 ml of CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). The anti-γ2 IgG was purified from a rabbit polyclonal antiserum prepared against a GST-fusion protein containing domain III of the γ2 chain (Pyke, C., Salo, S., Ralfkiaer. E., Romer, J., Dano, K. & Tryggvason, K. (1995) Cancer Res. 55: 4132-4139). The laminin-5 was eluted from the immunoaffinity

30   column using 50 mM triethanolamine, pH 11.25, 0.1% Triton X-100 and neutralized directly

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with 1 M Tris-HCl, pH 7.0. Collected fractions were analyzed by SDS-PAGE and Western blotting using the same polyclonal antibodies as used for the preparation of the affinity column. Fractions containing laminin-5 were pooled and dialyzed against 50 mM Tris-HCl, 0,1 M NaCl, pH 7.4. Some batches of laminin-5 were denatured with 5 M urea and renatured to study the effects of the treatment on adhesion and migration properties.

Generation of Recombinant Baculovirus and Expression of Recombinant Laminin 2 Chain

The γ2 chain of laminin-5 was expressed as recombinant protein using the baculovirus system and purified for studies on its functional properties. A full-length human laminin γ2 chain cDNA containing 6 bp of the 5' UTR and 822 bp of the 3' UTR was constructed from four overlapping cDNA clones L52, HT2-7, L15 and L61 (Kallunki, P., Sainio, K., Eddy, R., Byers, M., Kallunki, T., Sariola, H., Beck, K., Hirvonen, H., Shows, T.B. & Tryggvason, K. (1992) J. Cell Biol. 119: 679-693). The resulting 4,402 bp cDNA was analyzed by restriction enzyme mapping and partial sequencing, and cloned into the pVL1393 recombinant transfer plasmid prior to transfer into the AcNPV-γ2 baculovirus vector kindly provided by Max Summers (Texas A&M University). This baculovirus vector containing the human laminin γ2 chain cDNA under the transcriptional control of the polyhedrin promoter was produced and purified following standard procedures, except that it was first enriched according to the method of Pen, et al. (Pen, J., Welling, G.W. & Welling-Wester, S. (1989), Nucl. Acid. Res. 17: 451) from the virus containing medium obtained by co-transfecting Sf9 cells with the wild-type virus (AcNPV) DNA and the recombinant transfer vector pVL 1393-γ2. For expression of the recombinant protein, High Five (H5) cells were infected with the recombinant virus at a multiplicity of infection (MOI) of 5-10 pfu per cell by using standard protocols.

The recombinant γ2 chain was purified by first resuspending the cells in 10 volumes of 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2.5 mM EDTA, 1% Triton X-100, 1 mM PMSF and 1 mM NEM followed by homogenization in a Dounce homogenizer. The protein was extracted for 60 minutes on ice and solubilized proteins were removed by centrifugation at 1500 x g for 10 minutes at 4° C. The pellet was extracted again with buffer containing 1-3 M urea. The recombinant γ2 chain was extracted with a buffer containing 5 M urea, and renatured by dialysis against 50 mM Tris-HCl, pH 7.4, 100 mM NaCl.

Preparation of Antibodies

Polyclonal antiserum against domain III of the laminin γ2 chain was prepared and characterized as described previously. Briefly, rabbits were immunized s.c. four times using a γ2-GST fusion protein as antigen. The antigen contained 177 amino acid residues (res. # 391- 567) from domain III of the γ2 (**SEQ ID NO:8**) (Kallunki, P., Sainio, K., Eddy, R., Byers, M., Kallunki, T., Sariola, H., Beck, K., Hirvonen, H., Shows, T.B. & Tryggvason, K. (1992) J. Cell Biol. 119: 679-693). Antibodies against the GST-epitopes were removed from the antisera by negative immunoadsorption with GST-Sepharose made by coupling *E. coli* expressed GST protein to CNBr-activated Sepharore. The removal of anti-GST IgG was ensured by Western blotting analysis with GST-specific antibodies. The specificity of the antibody against the laminin γ2 chain was also tested by Western blotting as well as by ELISA.

Polyclonal antibody against the C-terminus of the laminin γ2 chain was produced in rabbits essentially as above for domain III using a γ2-GST fusion protein as antigen. The antigen contained 161 amino acids (res. # 1017-1178) from domain I/II of the γ2 chain and antibodies against the GST-epitopes were removed from the antisera by negative immunoadsorption with GST-Sepharose. The specificity of the antibody was tested by Western blotting and ELISA.

Polyclonal antiserum against laminin-1 was a kind gift of Dr. Foidart (University of Liege, Belgium). Normal rabbit serum was obtained prior to immunization from the rabbits used for immunization. IgG from the laminin-1 and laminin γ2 chain antisera, as well as from normal rabbit serum, was purified using Protein A Sepharose (Pharmacia, Uppsala, Sweden).

Cell Adhesion Assay

Microtiter plates (96 wells: Nunc, Copenhagen, Denmark) were coated with 100 µl/well of laminin-1 (10 µg/ml), laminin-5 (10 µg/ml), or recombinant laminin γ2 chain (10 µg/ml) in PBS or 50 M Tris-HCl, pH 7.4 by incubating the plates overnight at 4° C. Control wells were uncoated or coated with the same amounts of BSA. In some experiment the proteins were first denatured by dialysis overnight against 5 M urea, 50 mM Tris-HCl, pH 7.5 and then renatured by dialysis against 50 mM Tris-HCl, pH 7.5. Potential remaining active sites on the plates were blocked with 150 µl of 10 mg/ml BSA in PBS for 2 hours at room temperature. The wells were

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washed with PBS, and 100 ml of Eagle's MEM containing 5 mg/ml BSA was added. For the adhesion assays, KLN205 cells were detached from subconfluent cell culture dishes with trypsin-EDTA (0.25%-0.03%) and resuspended in Eagle's MEM/BSA (5 mg/ml) at a concentration of 2 x 10^5 cells/ml and allowed to recover for 20 minutes at 37° C. A total of 20,000 cells were then

5   added to each well and allowed to attach for an additional 90 minutes at 37° C. The extent of cell adhesion was determined by measuring color yields at 600 nm, following fixation with 3% paraformaldehyde and staining with 0.1% crystal violet. For inhibition assays with the anti-γ2 antibody, the substrate coated wells were incubated with 20 µg/ml of anti-γ2 chain IgG in PBS for 60 minutes prior to incubations with the cells.

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Migration Assay

The effect of endogenous laminin-5 on migration of KLN205 cells was determined by using a modified Boyden chamber assay, as described by Hujanen and Terranova (Hujanen, E. & Terranova, V.P. (1985) Cancer Res. 45: 3517-3521), and the effect of exogenous laminin-5 by

15   using a modified Transwell assay, as described by Pelletier, et al. (Pelletier, A.J., Kunicki, T. and Quaranta, V. (1996), J. Biol. Chem. 271:364).

The Boyden chamber assay was carried out as follows. Polycarbonate filters (pore size 10 µm, diameter 12 mm; Costar, Cambridge, MA) were coated with 2.5 µg of EHS type IV collagen, and used to separate the upper and lower compartments of the 50 µl chamber. A total

20   of 1 x 10^5 cells in Eagle's MEM containing 0.1% BSA were placed in the upper compartment, and the lower compartment was filled with medium with or without chemoattractants (50 µg/ml laminin-1 or fibronectin). To study the effect of the laminin γ2 chain antibodies on cell migration, anti-γ2 (III) IgG or anti-γ2 (C-term) IgG was added to the upper compartment together with the cells at a concentration of 20 µg/ml. Normal rabbit IgG was used as a negative

25   control. After an 8-hour incubation at 37° C in a humidified atmosphere, the filters were removed, fixed and stained (Diff-Quick, Baxter Diagnostics, Tubingen, Germany). The cells that had not migrated were removed from the upper surface of the filter with cotton swabs. Migration of cells was quantified by counting the cells on the lower surface of each filter in 10 randomly selected high power fields (x400). All assays were performed in triplicate.

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The "Transwell" plate assay (Transwell plates with pore size 12 µm, diameter 12 mm; Costar, Cambridge, MA) was used to determine the effect of exogenous laminin-5 on cell migration. The lower side of the membrane was coated with 2.5 µg of EHS type IV collagen for 3 hours at room temperature. Both sides were blocked with 1% bovine serum albumin for 1

5   hour. A total of 1 x 10^5 cells were added per well in the upper compartment in Eagle's MEM containing 10% FCS, and the lower compartment was filled with 2.5 µg/ml laminin-5 as a chemoattractant. Antibodies against the C-terminus and domain III of the γ2 chains or nonimmune IgG were added to the upper compartment, together with the cells at a concentration 20 µg/ml. Following a 16-hour incubation at 37° C the cells were fixed and stained. Cells on the

10   top surface of the membrane were removed with cotton swabs, and cells that had migrated to the lower side of the membrane were counted (12 fields +/- S.D.).

Immunohistochemical Staining

Five µm thick paraffin sections were stained with polyclonal antibodies against laminin-1

15   or the γ2 chain of laminin-5. In brief, the paraffin sections were first incubated with 0.4% pepsin in 0.1 M HCl at 37° C for 20 minutes to expose the antigens, blocked for nonspecific binding with 5% newborn rabbit serum, 0.1% BSA, and then incubated for 1 hour at 37° C with the polyclonal IgG diluted in TBS to 5-10 µg/ml. Subsequently, a biotinylated swine-anti-rabbit antibody was applied, followed by incubation with a 1:400 dilution of Horseradish-Peroxidase-

20   Avidin-Biotin-Complex (DAKO, Copenhagen, Denmark). The color was developed in diaminobentsamidine (DAB), followed by counterstaining of the slides with hematoxylin.

Results
Characterization of Proteins and Epithelium-Derived Cells

25   Immunopurified trimeric laminin-5, isolated from the culture medium of HaCat cells contained two major bands when analyzed by SDS-PAGE. These bands corresponded, respectively, to the 165 kDa γ2 chain, and the 155 kDa and 140 kDa γ2 and β3 chains migrating as a single band, as reported previously. Additionally, a weak band of about 105 kDa corresponding to the processed γ2 chain could be observed.

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Full-length human recombinant laminin γ2 chain was produced in High-5 *Spodoptera frugiperda* insect cells using the baculovirus system. Since the γ2 chain was not secreted to the culture medium, possibly because it was not assembled intracellularly into a normal heterotrimer, it was isolated from the cell fraction as described in *Materials and Methods*. The protein was

5   extracted under denaturing conditions using 5 M urea, renatured by extensive dialysis against 50 mM Tris-HCl, 100 mM NaCl, pH 7.4, and purified. The purified recombinant γ2 chain was full length (approximately 155 kDa) and highly pure as determined by SDS-PAGE analysis.

The HaCat human keratinocytes and mouse KLN205 squamous carcinoma cells were shown to express laminin-5, based on Northern blot analyses and immunostaining, using a

10   cDNA probe and/or polyclonal antibodies specific for the γ2 chain, respectively. Furthermore, the KLN205 cells developed γ2 chain positive primary tumors and metasases in mice *in vivo* (data not shown). Following intramuscular or subcutaneous inoculations, large primary tumors developed in 4 weeks with numerous lung metastases after 4-6 weeks. KLN205 cells injected into the tail vein produced multiple lung tumors (experimental metastases) in four weeks.

15   Consequently, both cell types were considered appropriate for the cell attachment and migration experiments carried out in this study.

Laminin-5 Molecule, but not Recombinant Laminin γ2 Chain, Promotes Cell Adhesion

The laminin-5 and recombinant γ2 chain prepared in this study, as well as commercial

20   laminin-1, were used as substrata in attachment assays (**FIGURE 1**) with the two epithelium-derived HaCat and KLN205 cell lines that both express laminin-5. Both cell lines attached about 2.5 times more readily to laminin-1 than to plastic. Adhesion of the cells to laminin-5 appeared to be slightly higher than that to laminin-1, but the differences were not statistically significant. The cells attached equally well to laminin-5 preparations denatured in 5 M urea and then

25   renatured by dialysis against 50 mM Tris-HCl, 100 mM NaCl, pH 7.4, as described for the recombinant γ2 chain above, indicating that this treatment did not affect the binding properties of the trimeric molecule. The attachment to laminin-5 did not significantly decrease in the presence of two different polyclonal antibodies made against the short or long arms of the γ2 chain or pre-IgG. Different amounts of the antibody against the short arm of the γ2 chain were also tested (up

30   to 50 μg/ml), but no effects on cell adhesion were observed. When the cells were plated on the

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recombinant γ2 chain alone, the attachment was not significantly higher than that to plastic, this attachment not being influenced by polyclonal antibodies against the γ2 chain. The data confirm previous results showing that trimeric laminin-5 promotes adhesion of epithelial cells, but the present results further strongly suggest that this adhesion is not mediated by the γ2 chain.

5

Antibodies Against Laminin γ2 Domain III, But Not Domain I/II, Inhibit Cell Migration

The potential role of the γ2 chain of laminin-5 in cell migration was examined for the KLN205 cells *in vitro* using Boyden and Transwell chamber assays as described in *Materials and Methods*.

10   Migration was first studied in the Boyden chamber assay using laminin-1 and fibronectin in the lower chamber as chemoattractants (See **Figure 2A**). The two compartments of the chemotactic Boyden chambers were separated by a type IV collagen coated porous filter (pore size 8 μm). The cells (1 x 10^5) in MEM containing 0.1% BSA were placed in the upper compartment, and laminin-1 (+/-) or fibronectin (-/+) in MEM containing 0.1% BSA were added

15 as chemoattractants to the lower compartment. IgG against γ2 chain domains III, I/II or preimmune IgG was added to the upper compartment with the cells at a concentration of 20 μg/ml. After an 8-hour incubation at 37°C the filters were removed and migration of cells to the lower surface of the filter was quantitated. The data are expressed as percentage of migrated cells (+/- SD (bars)) per high power field, setting migration in the presence of pre-immune IgG

20 as 100%. Cells were counted in ten randomly selected high power fields to triplicate assays. When polyclonal IgG against the short arm of the γ2 chain was added to the upper compartment containing the cells, the migration of cells through the filter was decreased to about 35 to 45% of that observed with the preimmune serum. In contrast, the polyclonal IgG against C-terminal domain I/II did not affect migration of the cells.

25   The effects of the two antibodies were similarly used in the Transwell assay using native laminin-5 as chemoattractant in the lower compartment (See **Figure 2B**). The lower side of the membrane was coated with EHS type IV collagen, and the lower compartment was filled with 2.5 μg/ml laminin-5 as a chemoattractant. Pre-immune IgG, IgG against the γ2 chain domains III or I/II were added to the upper chamber containing the cells. Following a 16-hour incubation

30 the cells were fixed and cells at the lower side of the membrane were counted (12 fields +/- SD).

The results were essentially the same as in the Boyden chamber assay. Thus, addition of IgG raised against domain III of the γ2 chain inhibited the migration to about 50% as compared with preimmune IgG, while the polyclonal IgG against domain I/II did not affect the cell migration.

These *in vitro* results demonstrate that laminin-5 have a role in the locomotion of
5   epithelium-derived cells, and that this function can be inhibited by antibodies directed against domain III of the γ2 chain.

Thus, antibodies against the short arm of the laminin λ2 chain inhibited the migration of KLN205 squamous carcinoma cells by about 55-65% as determined in the Boyden chamber migration assay. Interestingly, the antibodies used here were directed against 177 amino acid
10   residues of domain III (SEQ ID NO:8) that when deleted by mutation cause lethal junctional epidermolysis bullosa. Accordingly, the short arm of the laminin λ2 chain is important for the interaction of this laminin isoform to other extracellular matrix proteins and this interaction is also involved in the cell migration process.

15   EXAMPLE 2

The following example describes, in detail, the preparation of monoclonal antibodies according to the invention as well as demonstrating their use in inhibiting tumor cell growth in laminin-5 secreting tumors.

Monoclonal antibodies against the γ2 chain of laminin-5 were produced by immunizing
20   Balb/c mice with 100 ug GST-laminin-γ2-III fusion protein as antigen. The GST-laminin- γ2-III fusion protein contains human laminin- γ2-chain amino acid residues 391-567 (SEQ ID NO:8). Subsequent to immunization, spleen cells from the immunized mice were fused with mouse myeloma cell obtained from cell line P3X63Ag.8.653 (ATCC #CRL-1580). The hybridoma clones were then screened in immunohistology on frozen and paraffin sections (human cervix
25   carcinoma, normal cervix and normal skin) for the production of the anti-laminin- γ2 antibody. The staining result was compared to negative control, mouse normal serum and IgG, and to the positive result obtained with well-characterized anti-laminin-5, γ2 chain polyclonal antibody (described in Pyke, et al., 1995). The hybridoma clones were also screened in ELISA. The best hybridoma clones were picked and cloned again twice (single cell cloning) to ensure that the
30   produced hybridoma cell line was monoclonal.

The following describes the details of the production of three specific hybridoma clones

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and corresponding monoclonal antibodies produced therefrom. Characterization studies were conducted with respect to the 4G1, 5D5 and 6C12 monoclonal antibodies. Western blot analysis and ELISAs were carried out to confirm the specificity of the antibodies to the γ2 chain of laminin 5. Western blot analysis involved running recombinant laminin 5 γ2 chain (as well as
5   appropriate controls) in an SDS-PAGE gel, blotting the gel on a nylon membrane, and incubating the membrane with the antibodies

For ELISA, plates were coated with 100 μl GST-γ2-III fusion protein (antigen) (Salo et al., Matrix Biology 18:197-210 (1999) at a concentration of 2.5 ug/ml in 0.1M carbonate/bicarbonate buffer (pH 9) overnight at 4° C (0.25ug/well). The ELISA plate was then washed
10 three times with a PBST solution (200 μl) (10mM potassium phosphate, 150 mM NaCl), pH 7.5, and 0.05% Tween-20. Non-specific binding was then blocked by addition of BSA-PBS (1% bovine serum in PBS buffer (10mM K_3PO_4, 150 mM NaCl, pH 7.5)) (200 m/well) for a period of 90 minutes. To this, a dilution of negative controls (normal mouse serum) and a sample diluted in BSA-PBS (Mab 4G1, 5D5 or 6C12) were added and then the ELISA plate was incubated for 1
15 hour at room temperature. After incubation, the ELISA plate was then washed with PBST three times. Next, HRP-conjugated anti-mouse IgG secondary antibody (Peroxidase (HRP) conjugated Rabbit Anti-Mouse IgG (H+L), Jackson Laboratories #315-035-045) was added and the plate was incubated at room temperature for 30 minutes. The ELISA plate was then washed again three times with PBST solution (200 ml). An ABTS-peroxide substrate was then added to the
20 wells (ABTS diluted in 0.1 M Na-citrate, pH5; diluted immediately before assay use 1ml to 10ml with Na citrate buffer + 2 μl 30% hydrogen peroxide) and then the plate was allowed to incubate in the dark for 30 minutes. The absorbance was then read with a micro plate reader at 405 nm at 30 and 60 minutes.

These analyses demonstrated the specificity of the monoclonal antibodies for domain III
25 of the laminin 5 γ2 chain. Epitope mapping of the epitopes recognized by Mab 4G1, 5D5 or 6C12 indicated that they each bound epitopes within the amino acid sequence of **SEQ ID NO:6** (which is a portion of domain III of the γ2 chain that lacks part of the amino and carboxy terminal portions of domain III), and did not bind to epitopes within the amino acid sequence of **SEQ ID NOS: 9 and 10** (the 9 amino terminal and 41 carboxy terminal amino acids of domain
30 III, respectively).

17

Monoclonal antibodies against the γ2 chain of laminin-5 were then tested for efficacy in inhibiting tumor cell growth in laminin-5 secreting tumors.

Study 1:   Tumor Growth in Immunosuppressed Mice

5   The following study demonstrates the ability of IgG immunogloben against human laminin-5, γ2-III-domain (Mab 5D5) to affect the number and size of metastases in immune deficient mice.

10^6 human squamous epithelial carcinoma cells were injected into the tail vein of immunosuppressed mice for tumor implantation. The cell lines used were human squamous

10   epithelial carcinoma cells, cell line A431 and HSC-3. The cells were provided in suspensions in a medium containing DMEM-glutamax, 1% penicillin-streptomycin, 1% Na-pyruvate, 5% FCS. The cells were re-suspended in sterile Ca and Mg free PBS for inoculation. A control cell count was performed for the cell suspension at arrival and the cell density and the injected volume was recorded. The origin of the cells is HSC-3: Japan Health Science Research Resources Bank,

15   JCRB 0623 A431: ATCC catalog number CRL-1555. The immunosuppressed mice were selected as they are susceptible to grow cells of human origin as is well known in the field. The tumor cells in groups 3 and 6 were injected into mice with test item (test item was 50 µg/ml) for tumor implantation. The tumor cells were allowed to grow for one week after which the animal received intravenous injections of the test item twice a week for four weeks.

20

Table 1. Study Layout

Group	Mouse Strain		Animal Number	Cell Line	Treatment
1	Balb/c~nudet	5	1-5	–	–control, no treatment
2	Balb/c-nude[1]	5	6-10	HSC-3	+control, no treatment
3	Balb/c-nude[1]	5	1 1-15	HSC-3	Test item treatment: 50 µg 5D5l/mouse injection
4	SCID[2]	5	21-25	–	–control, no treatment
5	SCID~	5	26-30	A431	+control, no test item
6	SCIDZ	5	31-35	A431	Test item treatment: 50 µg 5D5/mouse injection

[1]   Balb/c-nude (BALBicABom-nu, M&B A/S, Denmark)

[2]   Fox Chase Scid (C.B-17/Icr scid/scid, M&B A/S, Denmark) immunodeficient mice.

After the treatment period, the animals were killed and tissue samples were collected. Number and size of the tumors in different tissues were counted and compared.

5   *Test Items and Dosing Solutions*

The test item was IgG immunoglobin against human laminin-5, γ2-III-domain (Mab 5D5). The test item was produced with monoclonal hybridoma method *in vitro* as set forth above. The test item (Mab 5D5) was suspended in sterile phosphate buffered saline (PBS) with a concentration of 1 mg/ml. The vehicle was sterilized using a 0.2 um filter. The delivered test

10   item was diluted with sterile PBS 50:50 to give a dosing concentration of 500 µg/ml.

The test item was administered intravenously into the lateral tail vein of the immunosuppressed mice in a volume of 0.1 ml/animal. The dosing was twice a week on Mondays and Thursdays. The first dose of test item was administered one week after the induction of experimental metastasis.

15   After four weeks of treatment (eight doses of test item), the animals were killed by exsanguination with cardiac puncture in CO_2 anesthesia. Blood was collected and serum separated and frozen in -20° C. A gross necropsy was performed and the macroscopic signs were recorded with special attention to macroscopic tumor masses, which were calculated and measured if possible. The following organs/tissues were collected and weighed: lungs, lymph

20   nodes (cervical and mesenerial), liver, and spleen. The organs/tissues were rinsed in PBS and fixed in 4% phosphate buffered formalin.

Clinical Signs

Animal number 6 had a thickening of the tail from day 5 through the whole study. The

25   tail of animal number 11 turned dark/black after tumor cell inoculation and eventually turned necrotic. Half of the tail was missing from day 7 onward. No other treatment related clinical signs were recorded. One animal (number 8, group 2) was found dead on the morning of the day following tumor cell inoculation. Gross necropsy did not reveal any macroscopic changes. All other animals survived in good condition during the whole study.

30

Necropsy

19

The injected tumor cells induced tumor growth almost only in the lungs. Other tissues with macroscopic metastases include spleen, liver, small intestine, and preputial gland. The SCID mice had changes in the liver which might be of microbial origin. In the lungs, the metastases were so numerous and so small that it was impractical to calculate or measure

5   individual metastases.

The following Table 2 represents a summarization of the results of the mice treated from Table 1.

Table 2. Experimental Metastases in Lung

10

Group	Mouse Strain	N	Cell Line	Treatment	Number of Mice with Macroscopic Lung Metastases Observed
1	Balb/c-nude	5	-	-control, no treatment	-
2*	Balb/c-nude	5	HSC-3	+ control, no treatment	4/4 (full of mastastases)
3	Balb/c-nude	5	HSC-3	Test item treatment	1/5
4	SCID ~	5	-	-control, no treatment	-
5	SCID	5	A431	+control, no test item	3/5
6	SCID	5'	A431	Test item treatment	4/5

*   one mouse was dead at the end of the second study

As can be seen from Table 2 above, the treated Balb/c-nude mice had 1 of 5 mice with macroscopic lung metastases while 4 of 4 untreated control Balb/c-nude mice had macroscopic

15   lung metastases.

EXAMPLE 3

Monoclonal antibody 5D5 was tested against HT29 carcinomas in a tumor growth inhibition assay. The assay compared immunotherapy with 75 and 25 µg/mouse 5D5, qod x 15,

. 20   to conventional chemotherapy with 100 mg/kg CPT-11 (irinotecan/Campostar), qwk x 3.

Methods and Materials

Female nude athymic mice (Harlan) were 13 weeks of age on day 1 of the study. The

20

animals were fed *ad libitum* water (reverse osmosis, 1 ppm Cl) and the NIH 31 Modified and Irradiated Lab Diet® consisting of 18.0% protein, 5.0% fat, and 5.0% fiber. Mice were housed in static microisolators on a 12-hour light cycle at 21-22 ° C (70-72 ° F) and 40%-60% humidity.

5   *Tumor Implantation*

An HT29 carcinoma fragment (1 mm^3) was implanted subcutaneously in the flank region of each mouse. When the tumors reached a size ranging from 62.5-126 mg, the mice were sorted into five treatment groups to provide a group mean tumor weights of 84.2-85.5 mg. Estimated tumor weight was calculated using the formula:

10

$$\text{Tumor Weight (mg)} = \frac{w^2 \times l}{2}$$

Where w = width and l = length in mm of the HT29 carcinoma.

15

Dosing solutions of 5D5 and control IgG were prepared fresh daily by dilution with phosphate-buffered saline. CPT-11 (Pharmacia; 20 mg/mL) was diluted with saline on each day of dosing.

On day 1, mice were sorted into five groups of animals (n = 10/group), and dosing was
20   initiated according to the protocols listed in Table 3.

Table 3. Protocol Design for the HT29-e29 Study

Group	n	Treatment Regimen 1			
		Agent	mg/kg	Route	Schedule
1	10	No treatment·	n/a		
2	10	CPT-11	100	IP	Qwkx3
3	10	Control IgG	75 ug/ mouse	IV	Qod x I 5
4	10	5D5	75 ug/ mouse	IV	Qod x I 5
5	10	5D5	25 ug/ mouse	IV	Qod x I 5~

As a positive reference drug, CPT- 11 was administered once per week for three weeks (qwk x 3) in 100 mg/kg doses. CPT- 11 was delivered i.p. in volumes of 0.2 ml/20 g body weight, which were body-weight adjusted. Doses of 5D5 or control mouse IgG were delivered intravenously in volumes of 0.2 mL/mouse. The antibody doses were not body-weight adjusted.

5 Untreated Group 1 mice served as controls for the CPT-11 therapy. Group 3 mice received 15 μg/mouse doses of control IgG once daily on alternate days (qod x 15). Mice in groups 4 and 5 received 75 and 25 ug/mouse doses of 5D5 x 15, respectively.

Endpoint

10 Efficacy was evaluated in a tumor growth inhibition assay. Tumors were measured twice weekly until the study was terminated on day 31. Each animal was then euthanized and its HT29 carcincoma was excised and weighed. Treatment may produce complete tumor regression (CR) or partial tumor regression (PR) in an animal. In a CR response, there is no measurable tumor mass at the completion of the study. In a PR response, the tumor weight is lower than the weight

15 on day, but greater than 0 mg. All tumors that did not regress were included in the calculation of tumor growth inhibition.

The increase in tumor weight for each animal was calculated as the difference between the actual tumor weight at the end of the study and the calculated tumor weight on day 1. These values were used to calculate the group mean tumor weight increases. Tumor growth inhibition

20 was calculated from the group mean tumor weight increases of treated and control mice by the following equation:

$$\%TGI = [\sim - (\underline{MeanNetTum\ orWeight_{Treated}})] \times 100\ \%$$
$$MeanNet\ Turn\ or\ Weight_{control}$$

25

Toxicity

The mice were weighed twice weekly until the end of the study. They were examined frequently for clinical signs of any adverse, drug-related side effects. Acceptable toxicity for cancer drugs in mice is defined by the NCI as a mean group weight loss of less than 20% during

30 the test, and not more than one toxic death among ten treated animals.

22

Statistics and Graphical Analyses

The unpaired t-test and Mann-Whitney U-test (for analysis of means and medians, respectively) were used to determine the statistical significance of the difference in mean tumor weights for mice in a treatment group and mice in a control group. The two-tailed statistical analyses were conducted at $P = 0.05$.

Results

Efficacy: Growth of HT29 Colon Carcinomas in Control Mice

Treatment protocols are listed in Table 3. Group 1 mice received no treatment and served as controls for CPT-11 and 5D5 therapy. Group 3 mice received fifteen 75 µg/mouse doses of irrelevant mouse IgG on alternate days (qod x 15). Table 4 summarizes the results for all groups in the study. The mean values for actual day 31 tumor weights in untreated and IgG-reated mice are 640.0 and 696.2 mg, respectively.

Table 4. Treatment Response Summary for the HT29-e28 Study

Gp.		Regimen 1				Final Tumor Weight Mean ± SEM (n)	Tumor Growth Inhibition	# CP	Mean % Tumor Decrease	# CR	Max. % BW Loss Day	# Death TR	
		Agent	mg/kg	Route	Schedule							TR	NTR
1	10	No treatment	n/a			640.0 ± 124.9 mg (10)	0%	0	None	0	-0.4%; Day 4	0	0
2	10	CPT-11	100	IP	Qwk x 3	447.9 ± 91.8 mg(10)	34.7%	0	None.	0	-5.8%; Day4	0	0
3	10	Control Igg	75 µg/ mouse	IV	Qod x 15	696.2 ± 131.4 mg (10)	0%	0.	None	0	-3.6%; Day 4	0	0
4	10	5D5	75µg/ mouse	IV	Qod x 15	543.5 ± 149.3 mg (8)	17.8%		None	0	-2.0%; Day 4	0	0
5	10	5D5	25µg/ mouse	IV	Qod x 15	700.7± 116.1 mg(10) ~	0%	0	None	0	-1.2%; Day4	0	0

ISDOCID: <WO____2004039401A2_I_>

Response of HT29 Xerographs to Intraperitoneal CPT-11 Therapy

Group 2 mice were treated once weekly for three weeks (qwk x 3) with i.p. injections' of 100
5   mg/kg CPT-11 (Table 3). No tumors regressed in response to CPT-11. The final mean tumor
weight in Group 3 mice was 447.9 mg (Table 4). Group 2 mice experienced 34.7% tumor growth
inhibition, relative to the untreated mice. This result, which is illustrated in a bar graph in **FIGURE**
3, was not statistically significant *(P* = 0.23.11, unpaired two-tailed t-test). **FIGURES 4A-E** shows
the growth of individual tumors in all treatment groups, as calculated from caliper measurements.
10   CPT-11 treatment caused a decrease in the slope of tumor growth.

Response of HT29 Xenografts to Intravenous 5D5 Immunotherapy

5D5 was administered intravenously to mice in Groups 4 and 5 on the qod x 15 schedule at
75 and 25 ug/mouse, respectively (Table 3). No tumor regressions were observed. The 75 and 25
15   µg/mg mouse 5D5 treatments yielded final actual mean tumor weights of 543.5 and 700.7,
respectively (Table 4). The high dose of 5D5 inhibited HT29 carcinoma growth by 17.8%, relative
to tumor growth in untreated mice. Tumor growth inhibition in Group 4 mice, relative to untreated
and IgG-treated mice, was not statistically significant *(P* = 0.6241 and 0.453, respectively; t-test).
Group 5 mice experienced no inhibition of tumor growth. **FIGURE 3** illustrates the lack of
20   significant tumor growth inhibition, given the large error (SEM) bars. **FIGURES 4 A-E** shows that
there was a modest decrease in the slopes of the tumor growth curves in animals treated with 75
ug/mouse 5D5.

Side Effects

25   All therapies were well tolerated. The highest group mean body-weight loss, an acceptable
5.8%, was recorded in mice treated with CPT-11. Body weight losses in antibody-treated mice were
3.6% or lower.

Discussion

30   The HT29 colon carcinoma xenograft model was appropriate for 5D5 evaluation because
HT29 cells produce laminin. Growth of primary tumors can be impeded by anti-proliferative agents,
such as CPT-11, as well as by agents that prevent invasion of the substratum. Combinational

treatments using monoclonal antibodies against the γ2 chain of laminin-5, such as 5D5, with anti-proliferative agents such as CPT-11 are also contemplated as part of the invention. Treatment efficacy was based on tumor growth inhibition, i.e., the difference between the mean increase in tumor size in control and treated groups of animals during the 31-day study. Although there was no
5   response to 5D5 at a dose of 25 µg/mouse, tumor growth was inhibited by 17.8% at 75 µg/mouse (Table 4 and **FIGURE 3**). Thus, a 75 µg/mouse dose of 5D5 produced some therapeutic effect against HT29 colon carcinomas. In general, there was a reduction in the slopes of the tumor growth curves in mice treated with CPT-11 and 5D5 (**FIGURES 4A-E**). Accordingly, these results indicate that anti-laminin immunotherapy has application in cancer treatment of laminin-5 secreting tumors.
10   In summary, established HT29 colon carcinomas responded to therapy with 75 µg/mouse doses of 5D5. High dose 5D5 immunotherapy achieved 50% of the tumor growth inhibition that was produced by CPT-11 chemotherapy. The tumor growth shown in **FIGURES 4A-E** curves suggest that 5D5 immunotherapy can impair colon tumor growth at doses of 75 pg/mouse or higher.

         Those skilled in the art will know, or be able to ascertain, using no more than routine
15   experimentation, many equivalents to the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

We claim:

1. Use of an antibody that binds to one or more epitopes of a laminin 5 γ2 chain for the preparation of a medicament for inhibiting tumor growth in a patient with a tumor.

2. The use of claim 1 wherein the antibody is a monoclonal antibody.

3. The use of claim 1 or 2 wherein the tumor is a carcinoma.

4. The use of any one of claims 1-3 wherein the medicament further comprises one or more further anti-tumor agent.

5. The use of claim 4 wherein the further anti-tumor agent is a chemotherapeutic agent.

6. An isolated antibody that binds to one or one or more epitopes of domain III of the laminin 5 γ2 chain but does not bind to epitopes within the amino acid sequence of **SEQ ID NOS: 9** and **10.**

7. The isolated antibody of claim 5 wherein the antibody is a monoclonal antibody.

8. A pharmaceutical composition comprising the isolated antibody of claim 5 and a pharmaceutically acceptable carrier.

9. Isolated hybridoma cells that express the monoclonal antibody of claim 6.

10. Use of the isolated antibody of any one of claims 6-8 for the preparation of a medicament for one or more of inhibiting tumor growth, inhibiting tumor metastasis, and detecting invasive cells in a patient with a tumor.

11. A pharmaceutical composition comprising an antibody that binds to one or more epitopes in domain III of laminin 5 γ2 chain and a further anti-tumor agent.

12. The pharmaceutical composition of claim 6 wherein the further anti-tumor agent is a chemotherapeutic agent.

13. A method for detecting the presence of invasive cells in a tissue comprising contacting the tissue sample with the antibody of any one of claims 6-8 to form an immunocomplex, and detecting formation of the immunocomplex, wherein the formation of the immunocomplex correlates with the presence of invasive cells in the tissue.

FIG. 1
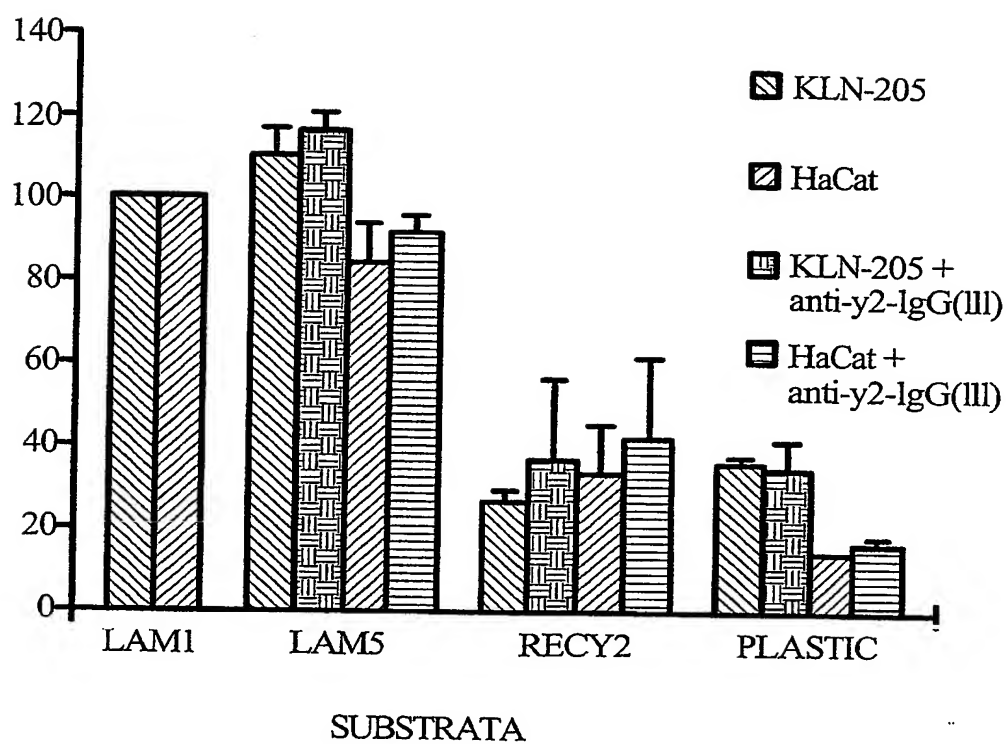
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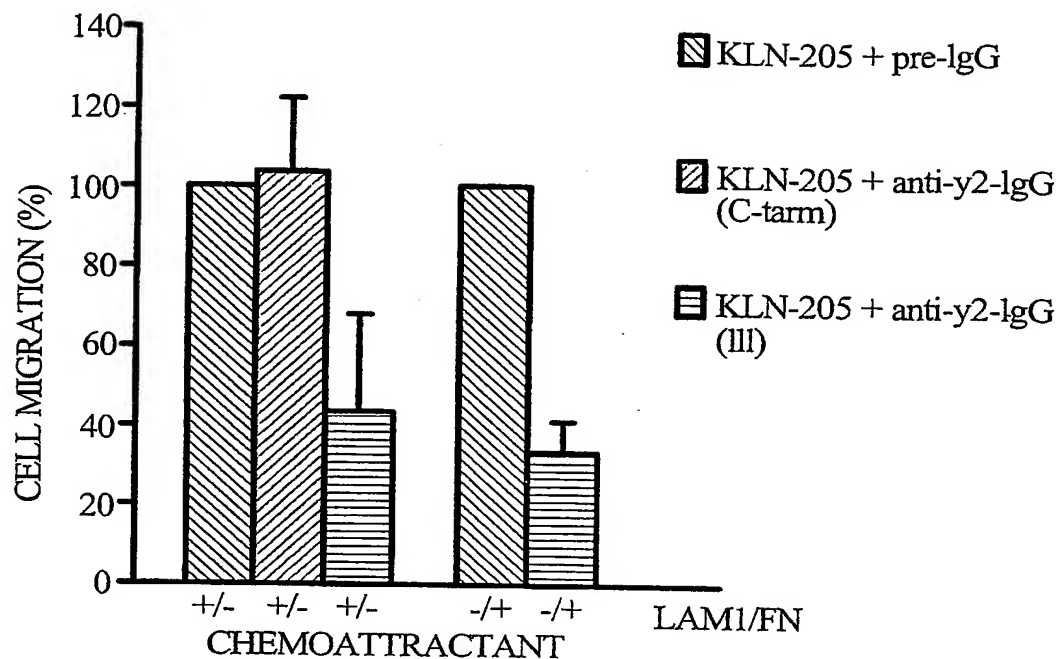
FIG. 2B
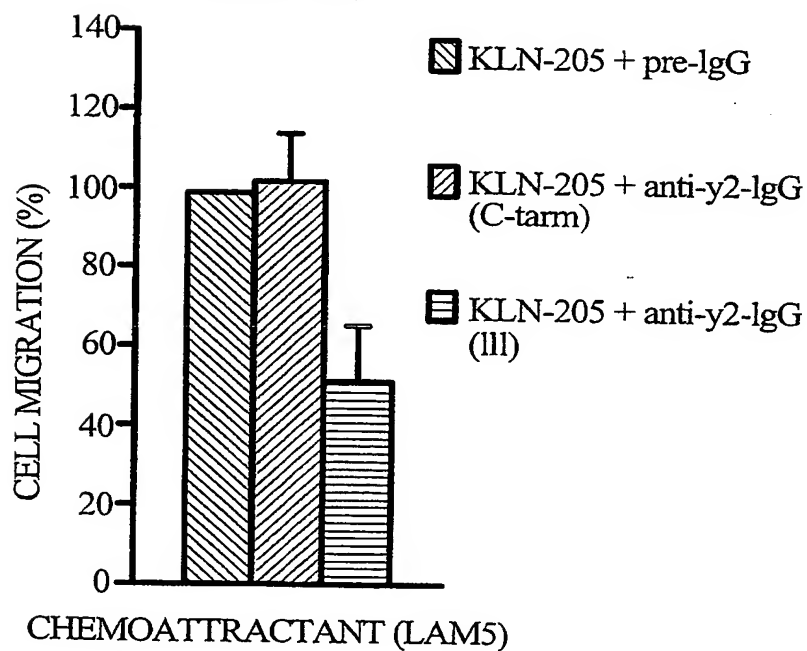
FIG. 3

TUMOR GROWTH INHIBITION ON DAY 31
IN THE HT29-e28 STUDY
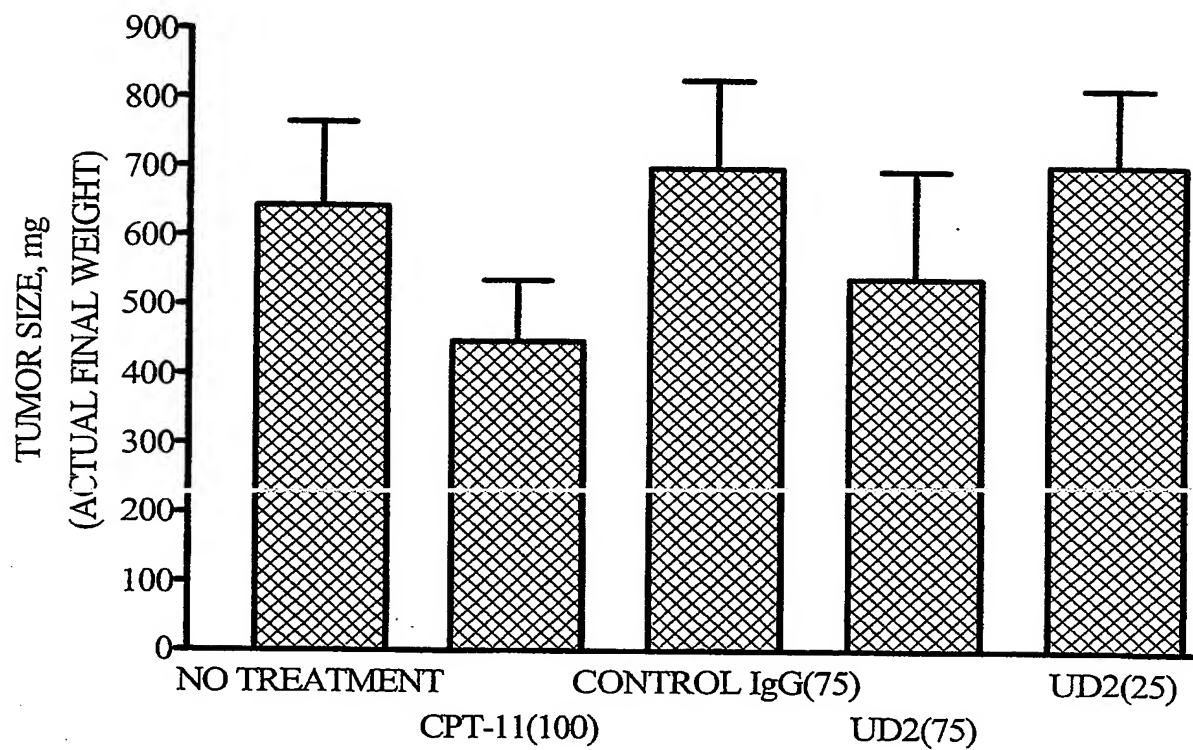
Tumor Growth Curves for Individual
Mice in the HT29-e28 Study
Group 1: No Treatment

Mouse ID
1 2 3 4 5 6 7 8 9 10

Study Cutoff
Size = 1500 mg

Days

Tumor Size, mg
(Caliper Estimate)
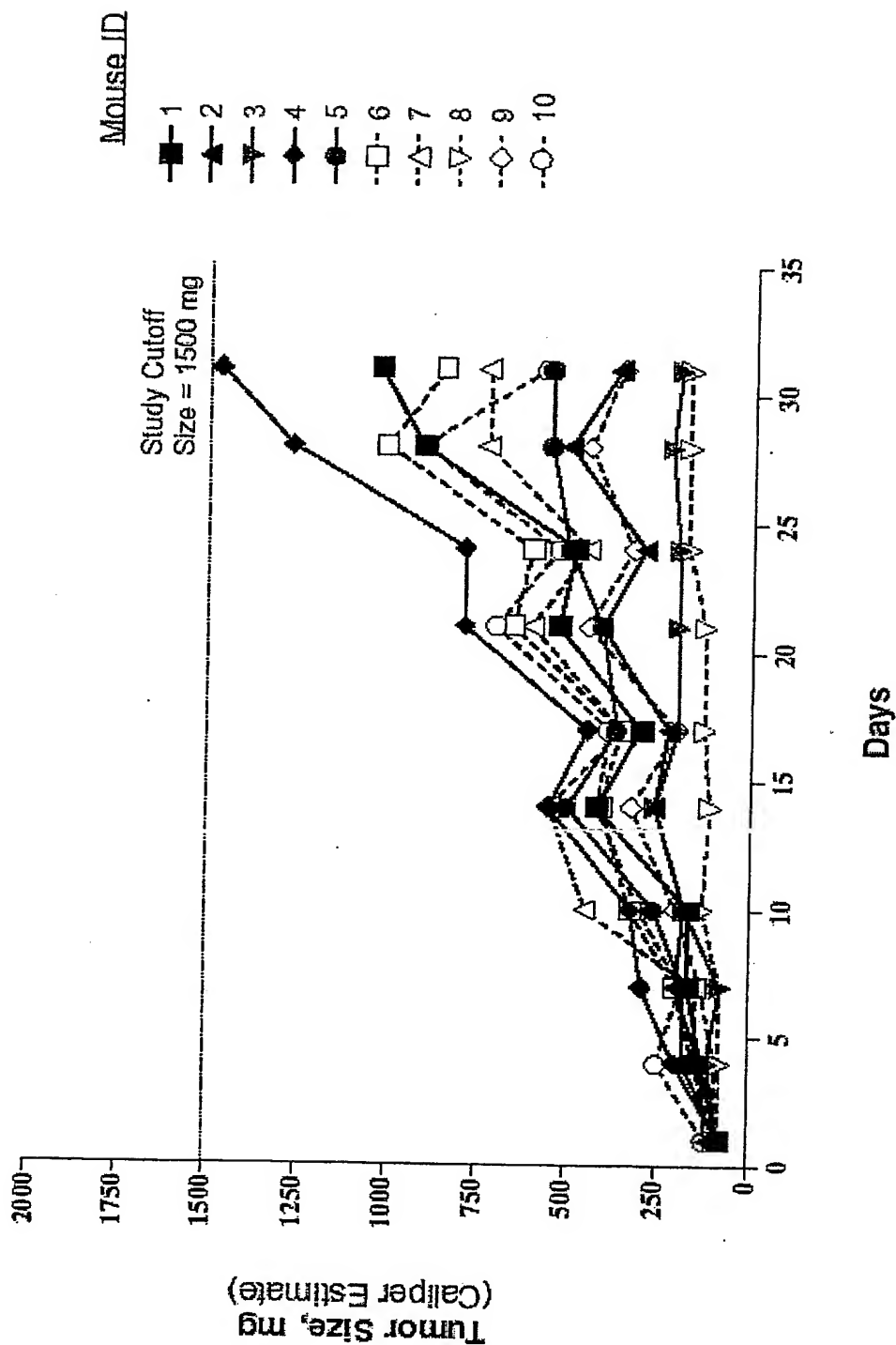
FIGURE 4A

Tumor Growth Curves for Individual
Mice in the HT29-e28 Study
Group 2: CPT-11: 100 mg/kg; ip; qwk x 3
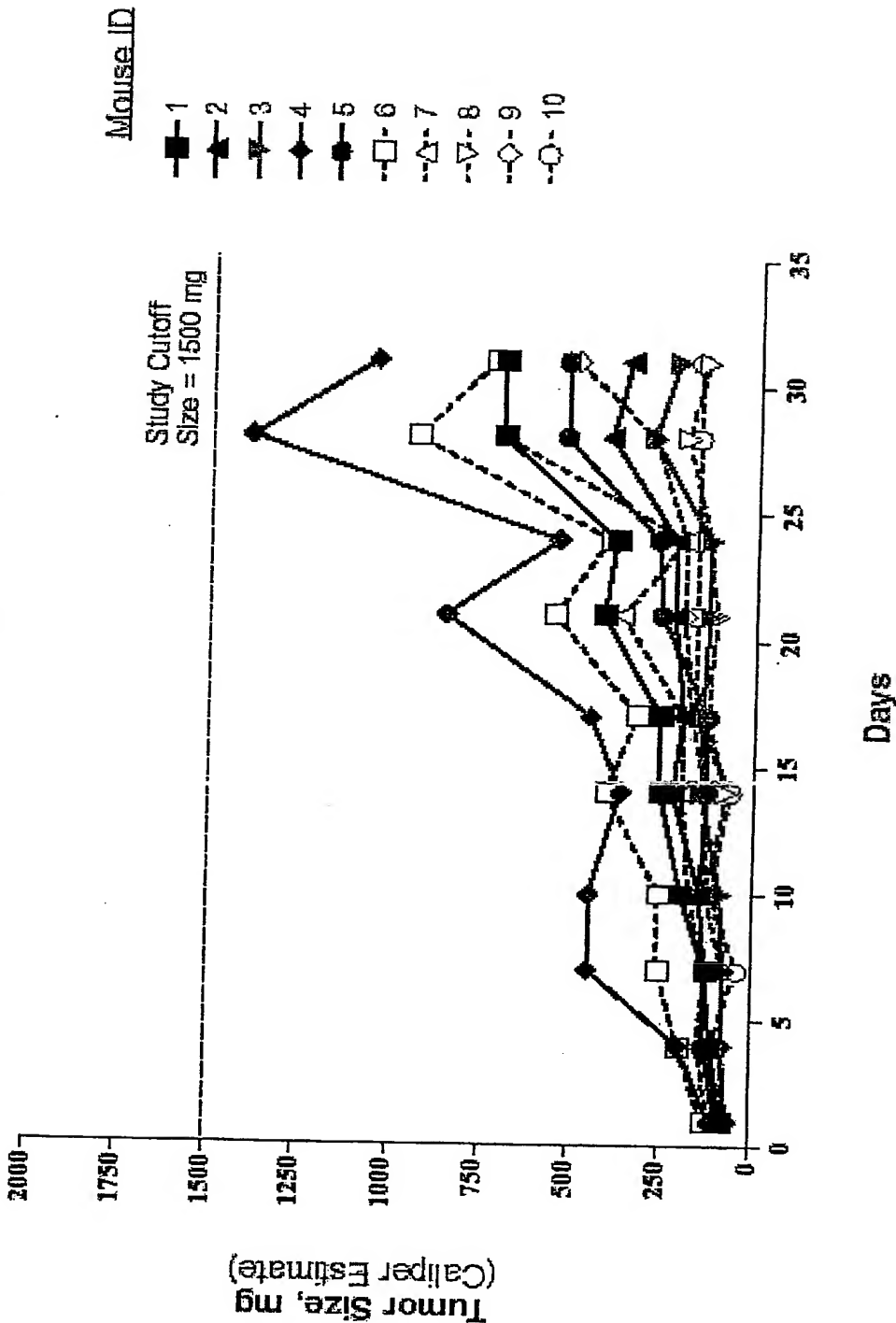
FIGURE 4B

Tumor Growth Curves for Individual
Mice in the HT29-e28 Study
Group 3: Control IgG; 75 mg/kg; iv; qod x15

FIGURE 4C

Tumor Growth Curves for Individual
Mice in the HT29-e28 Study
Group 4; UI/2; 75 mg/kg; iv; qod x 15
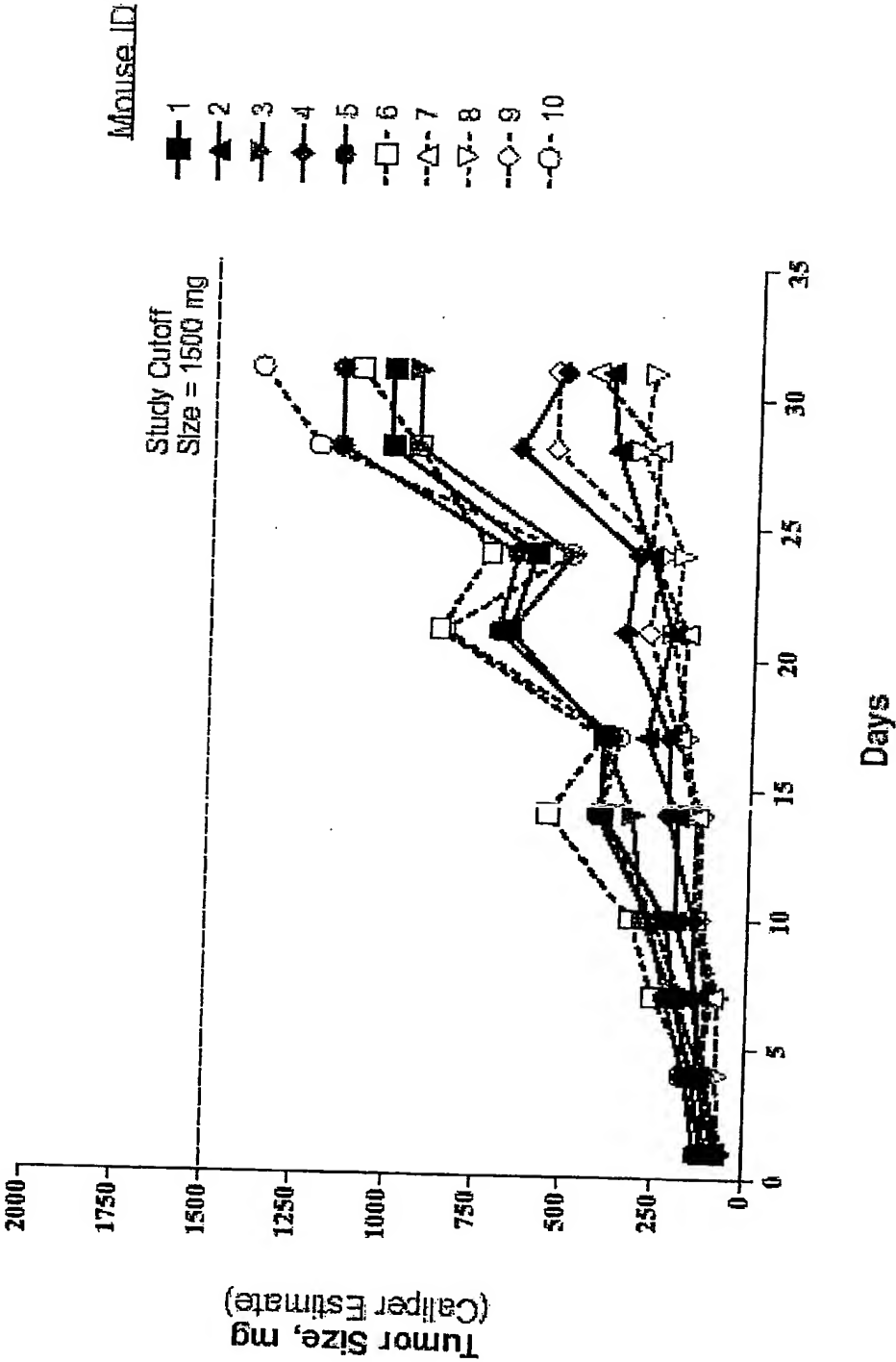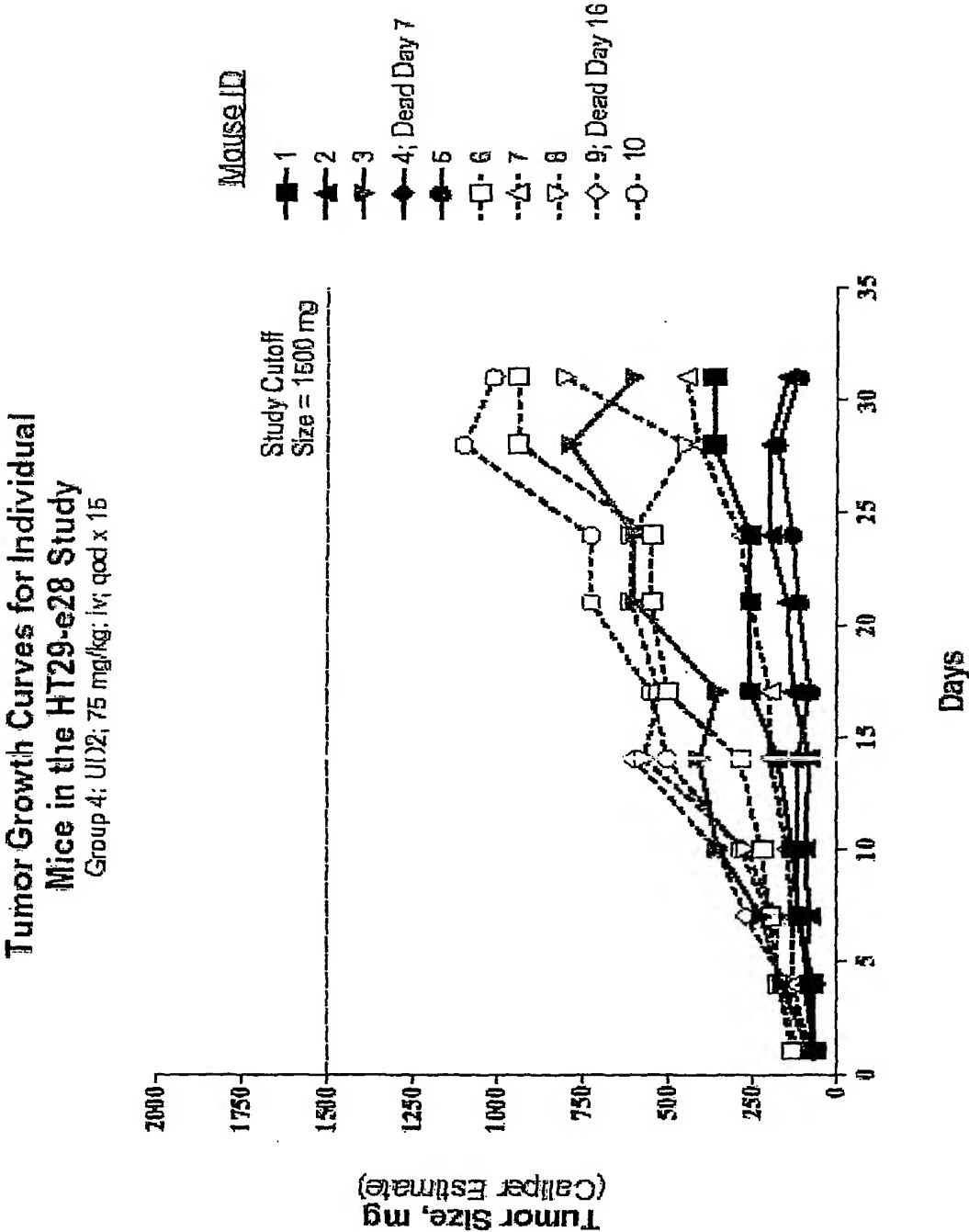
FIGURE 4D

Tumor Growth Curves for Individual
Mice in the HT29-e28 Study
Group 5: UD2; 25 mg/kg; iv; qod x15
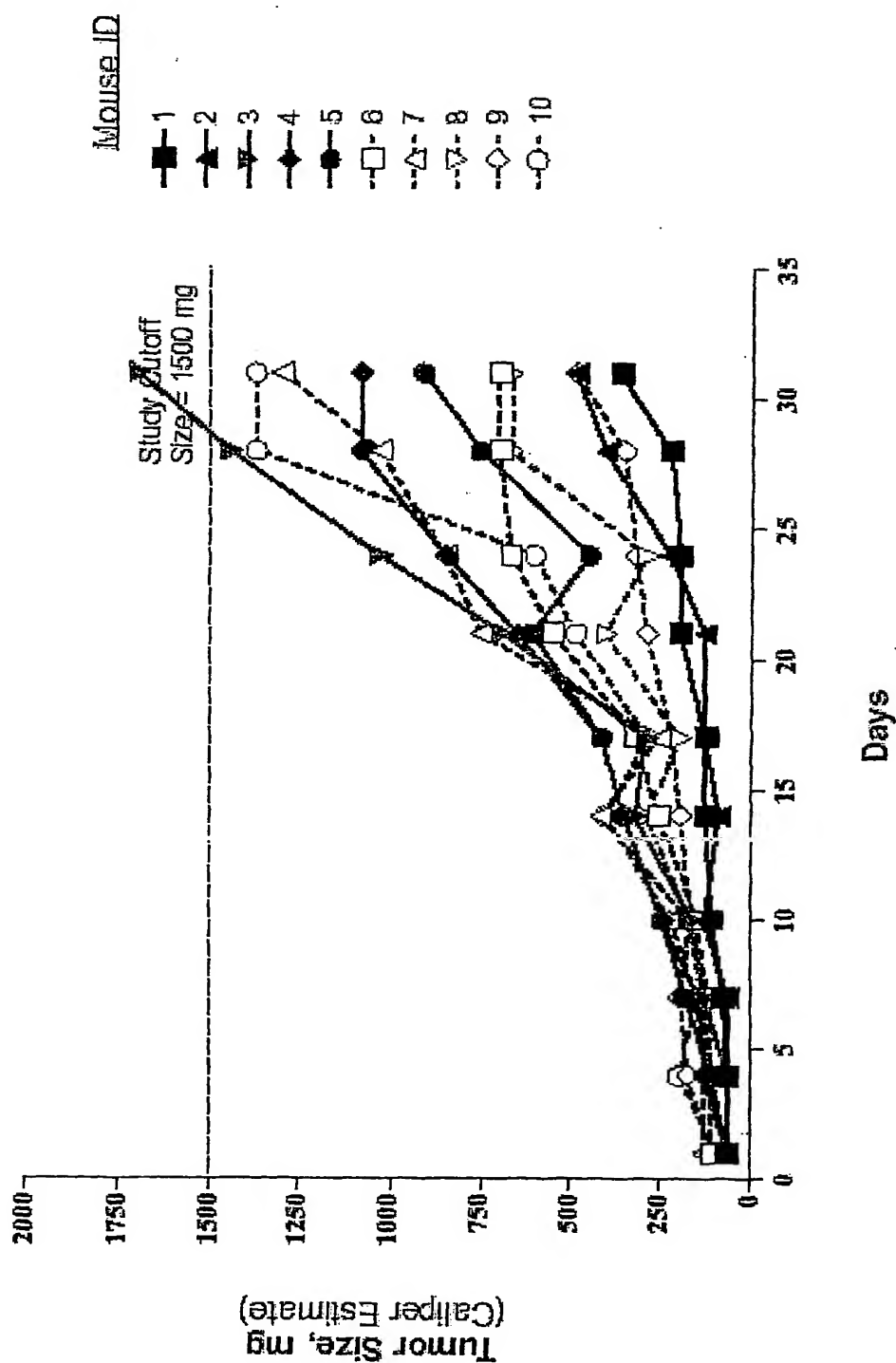
FIGURE 4E

SEQUENCE LISTING

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        Salo, Sirpa

<120> Use of antibodies to the gamma 2 chain of laminin 5 to inhibit tumor
growth and metastasis

<130> 02-1147-PCT

<150> 60/422,009
<151> 2002-10-29

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1   5   10   15

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            20   25   30

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Lys Ser Arg Gln Cys Ile Phe Asp Arg Glu Leu His Arg Gln Thr Gly
        35   40   45

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Asn Gly Phe Arg Cys Leu Asn Cys Asn Asp Asn Thr Asp Gly Ile His
    50   55   60

tgc gag aag tgc aag aat ggc ttt tac cgg cac aga gaa agg gac cgc   357
Cys Glu Lys Cys Lys Asn Gly Phe Tyr Arg His Arg Glu Arg Asp Arg
65   70   75   80

tgt ttg ccc tgc aat tgt aac tcc aaa ggt tct ctt agt gct cga tgt   405
Cys Leu Pro Cys Asn Cys Asn Ser Lys Gly Ser Leu Ser Ala Arg Cys
                85   90   95

gac aac tct gga cgg tgc agc tgt aaa cca ggt gtg aca gga gcc aga   453
Asp Asn Ser Gly Arg Cys Ser Cys Lys Pro Gly Val Thr Gly Ala Arg

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              100                        105                        110

tgc gac cga tgt ctg cca ggc ttc cac atg ctc acg gat gcg ggg tgc        501
Cys Asp Arg Cys Leu Pro Gly Phe His Met Leu Thr Asp Ala Gly Cys
        115                 120                 125

acc caa gac cag aga ctg cta gac tcc aag tgt gac tgt gac cca gct        549
Thr Gln Asp Gln Arg Leu Leu Asp Ser Lys Cys Asp Cys Asp Pro Ala
    130                 135                 140

ggc atc gca ggg ccc tgt gac gcg ggc cgc tgt gtc tgc aag cca gct        597
Gly Ile Ala Gly Pro Cys Asp Ala Gly Arg Cys Val Cys Lys Pro Ala
145                 150                 155                 160

gtt act gga gaa cgc tgt gat agg tgt cga tca ggt tac tat aat ctg        645
Val Thr Gly Glu Arg Cys Asp Arg Cys Arg Ser Gly Tyr Tyr Asn Leu
                165                 170                 175

gat ggg ggg aac cct gag ggc tgt acc cag tgt ttc tgc tat ggg cat        693
Asp Gly Gly Asn Pro Glu Gly Cys Thr Gln Cys Phe Cys Tyr Gly His
            180                 185                 190

tca gcc agc tgc cgc agc tct gca gaa tac agt gtc cat aag atc acc        741
Ser Ala Ser Cys Arg Ser Ser Ala Glu Tyr Ser Val His Lys Ile Thr
        195                 200                 205

tct acc ttt cat caa gat gtt gat ggc tgg aag gct gtc caa cga aat        789
Ser Thr Phe His Gln Asp Val Asp Gly Trp Lys Ala Val Gln Arg Asn
    210                 215                 220

ggg tct cct gca aag ctc caa tgg tca cag cgc cat caa gat gtg ttt        837
Gly Ser Pro Ala Lys Leu Gln Trp Ser Gln Arg His Gln Asp Val Phe
225                 230                 235                 240

agc tca gcc caa cga cta gat cct gtc tat ttt gtg gct cct gcc aaa        885
Ser Ser Ala Gln Arg Leu Asp Pro Val Tyr Phe Val Ala Pro Ala Lys
                245                 250                 255

ttt ctt ggg aat caa cag gtg agc tat ggg caa agc ctg tcc ttt gac        933
Phe Leu Gly Asn Gln Gln Val Ser Tyr Gly Gln Ser Leu Ser Phe Asp
            260                 265                 270

tac cgt gtg gac aga gga ggc aga cac cca tct gcc cat gat gtg atc        981
Tyr Arg Val Asp Arg Gly Gly Arg His Pro Ser Ala His Asp Val Ile
        275                 280                 285

ctg gaa ggt gct ggt cta cgg atc aca gct ccc ttg atg cca ctt ggc       1029
Leu Glu Gly Ala Gly Leu Arg Ile Thr Ala Pro Leu Met Pro Leu Gly
    290                 295                 300

aag aca ctg cct tgt ggg ctc acc aag act tac aca ttc agg tta aat       1077
Lys Thr Leu Pro Cys Gly Leu Thr Lys Thr Tyr Thr Phe Arg Leu Asn
305                 310                 315                 320

gag cat cca agc aat aat tgg agc ccc cag ctg agt tac ttt gag tat       1125
Glu His Pro Ser Asn Asn Trp Ser Pro Gln Leu Ser Tyr Phe Glu Tyr
                325                 330                 335

cga agg tta ctg cgg aat ctc aca gcc ctc cgc atc cga gct aca tat       1173
Arg Arg Leu Leu Arg Asn Leu Thr Ala Leu Arg Ile Arg Ala Thr Tyr
            340                 345                 350

gga gaa tac agt act ggg tac att gac aat gtg acc ctg att tca gcc       1221
Gly Glu Tyr Ser Thr Gly Tyr Ile Asp Asn Val Thr Leu Ile Ser Ala
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Gly Glu Tyr Ser Thr Gly Tyr Ile Asp Asn Val Thr Leu Ile Ser Ala
    355                 360             365

cgc cct gtc tct gga gcc cca gca ccc tgg gtt gaa cag tgt ata tgt    1269
Arg Pro Val Ser Gly Ala Pro Ala Pro Trp Val Glu Gln Cys Ile Cys
    370             375             380

cct gtt ggg tac aag ggg caa ttc tgc cag gat tgt gct tct ggc tac    1317
Pro Val Gly Tyr Lys Gly Gln Phe Cys Gln Asp Cys Ala Ser Gly Tyr
385             390             395             400

aag aga gat tca gcg aga ctg ggg cct ttt ggc acc tgt att cct tgt    1365
Lys Arg Asp Ser Ala Arg Leu Gly Pro Phe Gly Thr Cys Ile Pro Cys
                405             410             415

aac tgt caa ggg gga ggg gcc tgt gat cca gac aca gga gat tgt tat    1413
Asn Cys Gln Gly Gly Gly Ala Cys Asp Pro Asp Thr Gly Asp Cys Tyr
            420             425             430

tca ggg gat gag aat cct gac att gag tgt gct gac tgc cca att ggt    1461
Ser Gly Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly
            435             440             445

ttc tac aac gat ccg cac gac ccc cgc agc tgc aag cca tgt ccc tgt    1509
Phe Tyr Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys
    450             455             460

cat aac ggg ttc agc tgc tca gtg att ccg gag acg gag gag gtg gtg    1557
His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val
465             470             475             480

tgc aat aac tgc cct ccc ggg gtc acc ggt gcc cgc tgt gag ctc tgt    1605
Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys
            485             490             495

gct gat ggc tac ttt ggg gac ccc ttt ggt gaa cat ggc cca gtg agg    1653
Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg
            500             505             510

cct tgt cag ccc tgt caa tgc aac agc aat gtg gac ccc agt gcc tct    1701
Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser
        515             520             525

ggg aat tgt gac cgg ctg aca ggc agg tgt ttg aag tgt atc cac aac    1749
Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn
    530             535             540

aca gcc ggc atc tac tgc gac cag tgc aaa gca ggc tac ttc ggg gac    1797
Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp
545             550             555             560

cca ttg gct ccc aac cca gca gac aag tgt cga gct tgc aac tgt aac    1845
Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn
            565             570             575

ccc atg ggc tca gag cct gta gga tgt cga agt gat ggc acc tgt gtt    1893
Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr Cys Val
            580             585             590

tgc aag cca gga ttt ggt ggc ccc aac tgt gag cat gga gca ttc agc    1941
Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala Phe Ser
    595             600             605
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tgt cca gct tgc tat aat caa gtg aag att cag atg gat cag ttt atg    1989
Cys Pro Ala Cys Tyr Asn Gln Val Lys Ile Gln Met Asp Gln Phe Met
610             615             620

cag cag ctt cag aga atg gag gcc ctg att tca aag gct cag ggt ggt    2037
Gln Gln Leu Gln Arg Met Glu Ala Leu Ile Ser Lys Ala Gln Gly Gly
625             630             635             640

gat gga gta gta cct gat aca gag ctg gaa ggc agg atg cag cag gct    2085
Asp Gly Val Val Pro Asp Thr Glu Leu Glu Gly Arg Met Gln Gln Ala
                645             650             655

gag cag gcc ctt cag gac att ctg aga gat gcc cag att tca gaa ggt    2133
Glu Gln Ala Leu Gln Asp Ile Leu Arg Asp Ala Gln Ile Ser Glu Gly
                660             665             670

gct agc aga tcc ctt ggt ctc cag ttg gcc aag gtg agg agc caa gag    2181
Ala Ser Arg Ser Leu Gly Leu Gln Leu Ala Lys Val Arg Ser Gln Glu
        675             680             685

aac agc tac cag agc cgc ctg gat gac ctc aag atg act gtg gaa aga    2229
Asn Ser Tyr Gln Ser Arg Leu Asp Asp Leu Lys Met Thr Val Glu Arg
        690             695             700

gtt cgg gct ctg gga agt cag tac cag aac cga gtt cgg gat act cac    2277
Val Arg Ala Leu Gly Ser Gln Tyr Gln Asn Arg Val Arg Asp Thr His
705             710             715             720

agg ctc atc act cag atg cag ctg agc ctg gca gaa agt gaa gct tcc    2325
Arg Leu Ile Thr Gln Met Gln Leu Ser Leu Ala Glu Ser Glu Ala Ser
                725             730             735

ttg gga aac act aac att cct gcc tca gac cac tac gtg ggg cca aat    2373
Leu Gly Asn Thr Asn Ile Pro Ala Ser Asp His Tyr Val Gly Pro Asn
                740             745             750

ggc ttt aaa agt ctg gct cag gag gcc aca aga tta gca gaa agc cac    2421
Gly Phe Lys Ser Leu Ala Gln Glu Ala Thr Arg Leu Ala Glu Ser His
        755             760             765

gtt gag tca gcc agt aac atg gag caa ctg aca agg gaa act gag gac    2469
Val Glu Ser Ala Ser Asn Met Glu Gln Leu Thr Arg Glu Thr Glu Asp
770             775             780

tat tcc aaa caa gcc ctc tca ctg gtg cgc aag gcc ctg cat gaa gga    2517
Tyr Ser Lys Gln Ala Leu Ser Leu Val Arg Lys Ala Leu His Glu Gly
785             790             795             800

gtc gga agc gga agc ggt agc ccg gac ggt gct gtg gtg caa ggg ctt    2565
Val Gly Ser Gly Ser Gly Ser Pro Asp Gly Ala Val Val Gln Gly Leu
                805             810             815

gtg gaa aaa ttg gag aaa acc aag tcc ctg gcc cag cag ttg aca agg    2613
Val Glu Lys Leu Glu Lys Thr Lys Ser Leu Ala Gln Gln Leu Thr Arg
            820             825             830

gag gcc act caa gcg gaa att gaa gca gat agg tct tat cag cac agt    2661
Glu Ala Thr Gln Ala Glu Ile Glu Ala Asp Arg Ser Tyr Gln His Ser
            835             840             845

ctc cgc ctc ctg gat tca gtg tct ccg ctt cag gga gtc agt gat cag    2709
Leu Arg Leu Leu Asp Ser Val Ser Pro Leu Gln Gly Val Ser Asp Gln
850             855             860
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tcc ttt cag gtg gaa gaa gca aag agg atc aaa caa aaa gcg gat tca       2757
Ser Phe Gln Val Glu Glu Ala Lys Arg Ile Lys Gln Lys Ala Asp Ser
865             870             875             880

ctc tca agc ctg gta acc agg cat atg gat gag ttc aag cgt aca caa       2805
Leu Ser Ser Leu Val Thr Arg His Met Asp Glu Phe Lys Arg Thr Gln
                885             890             895

aag aat ctg gga aac tgg aaa gaa gaa gca cag cag ctc tta cag aat       2853
Lys Asn Leu Gly Asn Trp Lys Glu Glu Ala Gln Gln Leu Leu Gln Asn
                900             905             910

gga aaa agt ggg aga gag aaa tca gat cag ctg ctt tcc cgt gcc aat       2901
Gly Lys Ser Gly Arg Glu Lys Ser Asp Gln Leu Leu Ser Arg Ala Asn
            915             920             925

ctt gct aaa agc aga gca caa gaa gca ctg agt atg ggc aat gcc act       2949
Leu Ala Lys Ser Arg Ala Gln Glu Ala Leu Ser Met Gly Asn Ala Thr
        930             935             940

ttt tat gaa gtt gag agc atc ctt aaa aac ctc aga gag ttt gac ctg       2997
Phe Tyr Glu Val Glu Ser Ile Leu Lys Asn Leu Arg Glu Phe Asp Leu
945             950             955             960

cag gtg gac aac aga aaa gca gaa gct gaa gaa gcc atg aag aga ctc       3045
Gln Val Asp Asn Arg Lys Ala Glu Ala Glu Glu Ala Met Lys Arg Leu
            965             970             975

tcc tac atc agc cag aag gtt tca gat gcc agt gac aag acc cag caa       3093
Ser Tyr Ile Ser Gln Lys Val Ser Asp Ala Ser Asp Lys Thr Gln Gln
            980             985             990

gca gaa aga gcc ctg ggg agc gct gct gct gat gca cag agg gca aag       3141
Ala Glu Arg Ala Leu Gly Ser Ala Ala Ala Asp Ala Gln Arg Ala Lys
            995             1000            1005

aat ggg gcc ggg gag gcc ctg gaa atc tcc agt gag att gaa cag          3186
Asn Gly Ala Gly Glu Ala Leu Glu Ile Ser Ser Glu Ile Glu Gln
    1010            1015            1020

gag att ggg agt ctg aac ttg gaa gcc aat gtg aca gca gat gga          3231
Glu Ile Gly Ser Leu Asn Leu Glu Ala Asn Val Thr Ala Asp Gly
    1025            1030            1035

gcc ttg gcc atg gaa aag gga ctg gcc tct ctg aag agt gag atg          3276
Ala Leu Ala Met Glu Lys Gly Leu Ala Ser Leu Lys Ser Glu Met
    1040            1045            1050

agg gaa gtg gaa gga gag ctg gaa agg aag gag ctg gag ttt gac          3321
Arg Glu Val Glu Gly Glu Leu Glu Arg Lys Glu Leu Glu Phe Asp
    1055            1060            1065

acg aat atg gat gca gta cag atg gtg att aca gaa gcc cag aag          3366
Thr Asn Met Asp Ala Val Gln Met Val Ile Thr Glu Ala Gln Lys
    1070            1075            1080

gtt gat acc aga gcc aag aac gct ggg gtt aca atc caa gac aca          3411
Val Asp Thr Arg Ala Lys Asn Ala Gly Val Thr Ile Gln Asp Thr
    1085            1090            1095

ctc aac aca tta gac ggc ctc ctg cat ctg atg gac cag cct ctc          3456
Leu Asn Thr Leu Asp Gly Leu Leu His Leu Met Asp Gln Pro Leu
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agt gta gat gaa gag ggg ctg gtc tta ctg gag cag aag ctt tcc      3501
Ser Val Asp Glu Glu Gly Leu Val Leu Leu Glu Gln Lys Leu Ser
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cga gcc aag acc cag atc aac agc caa ctg cgg ccc atg atg tca      3546
Arg Ala Lys Thr Gln Ile Asn Ser Gln Leu Arg Pro Met Met Ser
    1130                  1135                  1140

gag ctg gaa gag agg gca cgt cag cag agg ggc cac ctc cat ttg      3591
Glu Leu Glu Glu Arg Ala Arg Gln Gln Arg Gly His Leu His Leu
    1145                  1150                  1155

ctg gag aca agc ata gat ggg att ctg gct gat gtg aag aac ttg      3636
Leu Glu Thr Ser Ile Asp Gly Ile Leu Ala Asp Val Lys Asn Leu
    1160                  1165                  1170

gag aac att agg gac aac ctg ccc cca ggc tgc tac aat acc cag      3681
Glu Asn Ile Arg Asp Asn Leu Pro Pro Gly Cys Tyr Asn Thr Gln
    1175                  1180                  1185

gct ctt gag caa cag tga agctgccata aatatttctc aactgaggtt        3729
Ala Leu Glu Gln Gln
    1190
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cttgggatac agatctcagg gctcgggagc catgtcatgt gagtgggtgg gatggggaca      3789

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ggccaggtgg ttgtcttatt gcaccatact ccttgcttcc tgatgctggg catgaggcag      3909

ataggcactg gtgtgagaat gatcaaggat ctggacccca agatagact  ggatggaaag      3969

acaaactgca caggcagatg tttgcctcat aatagtcgta agtggagtcc tggaatttgg      4029

acaagtgctg ttgggatata gtcaacttat tctttgagta atgtgactaa aggaaaaaac      4089

tttgactttg cccaggcatg aaattcttcc taatgtcaga acagagtgca acccagtcac      4149

actgtggcca gtaaaatact attgcctcat attgtcctct gcaagcttct tgctgatcag      4209

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gtgcctgcca ccttcaagtt ctggacctgg gcatgacatc ctttctttta atgatgccat      4389

ggcaacttag agattgcatt tttattaaag catttcctac cagcaaagca aatgttggga      4449

aagtatttac tttttcggtt tcaaagtgat agaaaagtgt ggcttgggca ttgaaagagg      4509

taaaattctc tagatttatt agtcctaatt caatcctact tttcgaacac caaaaatgat      4569

gcgcatcaat gtattttatc ttattttctc aatctcctct ctctttcctc cacccataat      4629

aagagaatgt tcctactcac acttcagctg ggtcacatcc atccctccat tcatccttcc      4689

atccatcttt ccatccatta cctccatcca tccttccaac atatatttat tgagtaccta      4749

ctgtgtgcca ggggctggtg ggacagtggt gacatagtct ctgccctcat agagttgatt      4809

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cacaagtggt gtttattgca ataaccgctt ggtttgcaac ctctttgctc aacagaacat    4929

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gcacatttct ttgcattcca gcgtcactct gtgccttcta caactgattg caacagactg    5049

ttgagttatg ataacaccag tgggaattgc tggaggaacc agaggcactt ccaccttggc    5109

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Lys Ser Arg Gln Cys Ile Phe Asp Arg Glu Leu His Arg Gln Thr Gly
        35                  40                  45


Asn Gly Phe Arg Cys Leu Asn Cys Asn Asp Asn Thr Asp Gly Ile His
    50                  55                  60


Cys Glu Lys Cys Lys Asn Gly Phe Tyr Arg His Arg Glu Arg Asp Arg
65                  70                  75                  80


Cys Leu Pro Cys Asn Cys Asn Ser Lys Gly Ser Leu Ser Ala Arg Cys
                85                  90                  95


Asp Asn Ser Gly Arg Cys Ser Cys Lys Pro Gly Val Thr Gly Ala Arg
            100                 105                 110


Cys Asp Arg Cys Leu Pro Gly Phe His Met Leu Thr Asp Ala Gly Cys
            115                 120                 125


Thr Gln Asp Gln Arg Leu Leu Asp Ser Lys Cys Asp Cys Asp Pro Ala
        130                 135                 140


Gly Ile Ala Gly Pro Cys Asp Ala Gly Arg Cys Val Cys Lys Pro Ala
145                 150                 155                 160


Val Thr Gly Glu Arg Cys Asp Arg Cys Arg Ser Gly Tyr Tyr Asn Leu
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Asp Gly Gly Asn Pro Glu Gly Cys Thr Gln Cys Phe Cys Tyr Gly His
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Ser Ala Ser Cys Arg Ser Ser Ala Glu Tyr Ser Val His Lys Ile Thr
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Ser Thr Phe His Gln Asp Val Asp Gly Trp Lys Ala Val Gln Arg Asn
    210   215   220

Gly Ser Pro Ala Lys Leu Gln Trp Ser Gln Arg His Gln Asp Val Phe
225   230   235   240

Ser Ser Ala Gln Arg Leu Asp Pro Val Tyr Phe Val Ala Pro Ala Lys
            245   250   255

Phe Leu Gly Asn Gln Gln Val Ser Tyr Gly Gln Ser Leu Ser Phe Asp
            260   265   270

Tyr Arg Val Asp Arg Gly Gly Arg His Pro Ser Ala His Asp Val Ile
    275   280   285

Leu Glu Gly Ala Gly Leu Arg Ile Thr Ala Pro Leu Met Pro Leu Gly
290   295   300

Lys Thr Leu Pro Cys Gly Leu Thr Lys Thr Tyr Thr Phe Arg Leu Asn
305   310   315   320

Glu His Pro Ser Asn Asn Trp Ser Pro Gln Leu Ser Tyr Phe Glu Tyr
            325   330   335

Arg Arg Leu Leu Arg Asn Leu Thr Ala Leu Arg Ile Arg Ala Thr Tyr
            340   345   350

Gly Glu Tyr Ser Thr Gly Tyr Ile Asp Asn Val Thr Leu Ile Ser Ala
            355   360   365

Arg Pro Val Ser Gly Ala Pro Ala Pro Trp Val Glu Gln Cys Ile Cys
    370   375   380

Pro Val Gly Tyr Lys Gly Gln Phe Cys Gln Asp Cys Ala Ser Gly Tyr
385   390   395   400

Lys Arg Asp Ser Ala Arg Leu Gly Pro Phe Gly Thr Cys Ile Pro Cys
            405   410   415

Asn Cys Gln Gly Gly Gly Ala Cys Asp Pro Asp Thr Gly Asp Cys Tyr

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                    420                      425                      430


Ser Gly Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly
        435                  440                  445


Phe Tyr Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys
        450                  455                  460


His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val
465                  470                  475                  480


Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys
                485                  490                  495


Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg
                500                  505                  510


Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser
        515                  520                  525


Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn
        530                  535                  540


Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp
545                  550                  555                  560


Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn
                565                  570                  575


Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr Cys Val
                580                  585                  590


Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala Phe Ser
        595                  600                  605


Cys Pro Ala Cys Tyr Asn Gln Val Lys Ile Gln Met Asp Gln Phe Met
        610                  615                  620


Gln Gln Leu Gln Arg Met Glu Ala Leu Ile Ser Lys Ala Gln Gly Gly
625                  630                  635                  640


Asp Gly Val Val Pro Asp Thr Glu Leu Glu Gly Arg Met Gln Gln Ala
                645                  650                  655


Glu Gln Ala Leu Gln Asp Ile Leu Arg Asp Ala Gln Ile Ser Glu Gly
                660                  665                  670
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Ala Ser Arg Ser Leu Gly Leu Gln Leu Ala Lys Val Arg Ser Gln Glu
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Asn Ser Tyr Gln Ser Arg Leu Asp Asp Leu Lys Met Thr Val Glu Arg
        690   695   700

Val Arg Ala Leu Gly Ser Gln Tyr Gln Asn Arg Val Arg Asp Thr His
705   710   715   720

Arg Leu Ile Thr Gln Met Gln Leu Ser Leu Ala Glu Ser Glu Ala Ser
                725   730   735

Leu Gly Asn Thr Asn Ile Pro Ala Ser Asp His Tyr Val Gly Pro Asn
                740   745   750

Gly Phe Lys Ser Leu Ala Gln Glu Ala Thr Arg Leu Ala Glu Ser His
        755   760   765

Val Glu Ser Ala Ser Asn Met Glu Gln Leu Thr Arg Glu Thr Glu Asp
        770   775   780

Tyr Ser Lys Gln Ala Leu Ser Leu Val Arg Lys Ala Leu His Glu Gly
785   790   795   800

Val Gly Ser Gly Ser Gly Ser Pro Asp Gly Ala Val Val Gln Gly Leu
                805   810   815

Val Glu Lys Leu Glu Lys Thr Lys Ser Leu Ala Gln Gln Leu Thr Arg
                820   825   830

Glu Ala Thr Gln Ala Glu Ile Glu Ala Asp Arg Ser Tyr Gln His Ser
        835   840   845

Leu Arg Leu Leu Asp Ser Val Ser Pro Leu Gln Gly Val Ser Asp Gln
850   855   860

Ser Phe Gln Val Glu Glu Ala Lys Arg Ile Lys Gln Lys Ala Asp Ser
865   870   875   880

Leu Ser Ser Leu Val Thr Arg His Met Asp Glu Phe Lys Arg Thr Gln
                885   890   895

Lys Asn Leu Gly Asn Trp Lys Glu Glu Ala Gln Gln Leu Leu Gln Asn
                900   905   910

Gly Lys Ser Gly Arg Glu Lys Ser Asp Gln Leu Leu Ser Arg Ala Asn
        915   920   925

Leu Ala Lys Ser Arg Ala Gln Glu Ala Leu Ser Met Gly Asn Ala Thr
930   935   940

Phe Tyr Glu Val Glu Ser Ile Leu Lys Asn Leu Arg Glu Phe Asp Leu
945   950   955   960

Gln Val Asp Asn Arg Lys Ala Glu Ala Glu Glu Ala Met Lys Arg Leu
                965   970   975

Ser Tyr Ile Ser Gln Lys Val Ser Asp Ala Ser Asp Lys Thr Gln Gln
            980   985   990

Ala Glu Arg Ala Leu Gly Ser Ala Ala Ala Asp Ala Gln Arg Ala Lys
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Asn Gly Ala Gly Glu Ala Leu Glu Ile Ser Ser Glu Ile Glu Gln
    1010   1015   1020

Glu Ile Gly Ser Leu Asn Leu Glu Ala Asn Val Thr Ala Asp Gly
    1025   1030   1035

Ala Leu Ala Met Glu Lys Gly Leu Ala Ser Leu Lys Ser Glu Met
    1040   1045   1050

Arg Glu Val Glu Gly Glu Leu Glu Arg Lys Glu Leu Glu Phe Asp
    1055   1060   1065

Thr Asn Met Asp Ala Val Gln Met Val Ile Thr Glu Ala Gln Lys
    1070   1075   1080

Val Asp Thr Arg Ala Lys Asn Ala Gly Val Thr Ile Gln Asp Thr
    1085   1090   1095

Leu Asn Thr Leu Asp Gly Leu Leu His Leu Met Asp Gln Pro Leu
    1100   1105   1110

Ser Val Asp Glu Glu Gly Leu Val Leu Leu Glu Gln Lys Leu Ser
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Arg Ala Lys Thr Gln Ile Asn Ser Gln Leu Arg Pro Met Met Ser
    1130   1135   1140

Glu Leu Glu Glu Arg Ala Arg Gln Gln Arg Gly His Leu His Leu
    1145   1150   1155

Leu Glu Thr Ser Ile Asp Gly Ile Leu Ala Asp Val Lys Asn Leu
    1160   1165   1170

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Ala Leu  Glu Gln Gln
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aggcgccggg cagcgacccc tgcagcggag acagagactg agcggcccgg caccgcc        117

atg cct gcg ctc tgg ctg ggc tgc tgc ctc tgc ttc tcg ctc ctc ctg      165
Met Pro Ala Leu Trp Leu Gly Cys Cys Leu Cys Phe Ser Leu Leu Leu
1               5                   10                  15

ccc gca gcc cgg gcc acc tcc agg agg gaa gtc tgt gat tgc aat ggg      213
Pro Ala Ala Arg Ala Thr Ser Arg Arg Glu Val Cys Asp Cys Asn Gly
            20                  25                  30

aag tcc agg cag tgt atc ttt gat cgg gaa ctt cac aga caa act ggt      261
Lys Ser Arg Gln Cys Ile Phe Asp Arg Glu Leu His Arg Gln Thr Gly
        35                  40                  45

aat gga ttc cgc tgc ctc aac tgc aat gac aac act gat ggc att cac      309
Asn Gly Phe Arg Cys Leu Asn Cys Asn Asp Asn Thr Asp Gly Ile His
    50                  55                  60

tgc gag aag tgc aag aat ggc ttt tac cgg cac aga gaa agg gac cgc      357
Cys Glu Lys Cys Lys Asn Gly Phe Tyr Arg His Arg Glu Arg Asp Arg
65                  70                  75                  80

tgt ttg ccc tgc aat tgt aac tcc aaa ggt tct ctt agt gct cga tgt      405
Cys Leu Pro Cys Asn Cys Asn Ser Lys Gly Ser Leu Ser Ala Arg Cys
                85                  90                  95
```

```
gac aac tct gga cgg tgc agc tgt aaa cca ggt gtg aca gga gcc aga      453
Asp Asn Ser Gly Arg Cys Ser Cys Lys Pro Gly Val Thr Gly Ala Arg
            100                 105                 110

tgc gac cga tgt ctg cca ggc ttc cac atg ctc acg gat gcg ggg tgc      501
Cys Asp Arg Cys Leu Pro Gly Phe His Met Leu Thr Asp Ala Gly Cys
            115                 120                 125

acc caa gac cag aga ctg cta gac tcc aag tgt gac tgt gac cca gct      549
Thr Gln Asp Gln Arg Leu Leu Asp Ser Lys Cys Asp Cys Asp Pro Ala
            130                 135                 140

ggc atc gca ggg ccc tgt gac gcg ggc cgc tgt gtc tgc aag cca gct      597
Gly Ile Ala Gly Pro Cys Asp Ala Gly Arg Cys Val Cys Lys Pro Ala
145                 150                 155                 160

gtt act gga gaa cgc tgt gat agg tgt cga tca ggt tac tat aat ctg      645
Val Thr Gly Glu Arg Cys Asp Arg Cys Arg Ser Gly Tyr Tyr Asn Leu
                165                 170                 175

gat ggg ggg aac cct gag ggc tgt acc cag tgt ttc tgc tat ggg cat      693
Asp Gly Gly Asn Pro Glu Gly Cys Thr Gln Cys Phe Cys Tyr Gly His
            180                 185                 190

tca gcc agc tgc cgc agc tct gca gaa tac agt gtc cat aag atc acc      741
Ser Ala Ser Cys Arg Ser Ser Ala Glu Tyr Ser Val His Lys Ile Thr
            195                 200                 205

tct acc ttt cat caa gat gtt gat ggc tgg aag gct gtc caa cga aat      789
Ser Thr Phe His Gln Asp Val Asp Gly Trp Lys Ala Val Gln Arg Asn
            210                 215                 220

ggg tct cct gca aag ctc caa tgg tca cag cgc cat caa gat gtg ttt      837
Gly Ser Pro Ala Lys Leu Gln Trp Ser Gln Arg His Gln Asp Val Phe
225                 230                 235                 240

agc tca gcc caa cga cta gat cct gtc tat ttt gtg gct cct gcc aaa      885
Ser Ser Ala Gln Arg Leu Asp Pro Val Tyr Phe Val Ala Pro Ala Lys
                245                 250                 255

ttt ctt ggg aat caa cag gtg agc tat ggg caa agc ctg tcc ttt gac      933
Phe Leu Gly Asn Gln Gln Val Ser Tyr Gly Gln Ser Leu Ser Phe Asp
            260                 265                 270

tac cgt gtg gac aga gga ggc aga cac cca tct gcc cat gat gtg atc      981
Tyr Arg Val Asp Arg Gly Gly Arg His Pro Ser Ala His Asp Val Ile
            275                 280                 285

ctg gaa ggt gct ggt cta cgg atc aca gct ccc ttg atg cca ctt ggc     1029
Leu Glu Gly Ala Gly Leu Arg Ile Thr Ala Pro Leu Met Pro Leu Gly
            290                 295                 300

aag aca ctg cct tgt ggg ctc acc aag act tac aca ttc agg tta aat     1077
Lys Thr Leu Pro Cys Gly Leu Thr Lys Thr Tyr Thr Phe Arg Leu Asn
305                 310                 315                 320

gag cat cca agc aat aat tgg agc ccc cag ctg agt tac ttt gag tat     1125
Glu His Pro Ser Asn Asn Trp Ser Pro Gln Leu Ser Tyr Phe Glu Tyr
                325                 330                 335

cga agg tta ctg cgg aat ctc aca gcc ctc cgc atc cga gct aca tat     1173
Arg Arg Leu Leu Arg Asn Leu Thr Ala Leu Arg Ile Arg Ala Thr Tyr
```

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                340                      345                      350

gga gaa tac agt act ggg tac att gac aat gtg acc ctg att tca gcc        1221
Gly Glu Tyr Ser Thr Gly Tyr Ile Asp Asn Val Thr Leu Ile Ser Ala
        355                      360                      365

cgc cct gtc tct gga gcc cca gca ccc tgg gtt gaa cag tgt ata tgt        1269
Arg Pro Val Ser Gly Ala Pro Ala Pro Trp Val Glu Gln Cys Ile Cys
        370                      375                      380

cct gtt ggg tac aag ggg caa ttc tgc cag gat tgt gct tct ggc tac        1317
Pro Val Gly Tyr Lys Gly Gln Phe Cys Gln Asp Cys Ala Ser Gly Tyr
385                      390                      395                  400

aag aga gat tca gcg aga ctg ggg cct ttt ggc acc tgt att cct tgt        1365
Lys Arg Asp Ser Ala Arg Leu Gly Pro Phe Gly Thr Cys Ile Pro Cys
                405                      410                      415

aac tgt caa ggg gga ggg gcc tgt gat cca gac aca gga gat tgt tat        1413
Asn Cys Gln Gly Gly Gly Ala Cys Asp Pro Asp Thr Gly Asp Cys Tyr
                420                      425                      430

tca ggg gat gag aat cct gac att gag tgt gct gac tgc cca att ggt        1461
Ser Gly Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly
                435                      440                      445

ttc tac aac gat ccg cac gac ccc cgc agc tgc aag cca tgt ccc tgt        1509
Phe Tyr Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys
        450                      455                      460

cat aac ggg ttc agc tgc tca gtg att ccg gag acg gag gag gtg gtg        1557
His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val
465                      470                      475                  480

tgc aat aac tgc cct ccc ggg gtc acc ggt gcc cgc tgt gag ctc tgt        1605
Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys
                485                      490                      495

gct gat ggc tac ttt ggg gac ccc ttt ggt gaa cat ggc cca gtg agg        1653
Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg
                500                      505                      510

cct tgt cag ccc tgt caa tgc aac agc aat gtg gac ccc agt gcc tct        1701
Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser
        515                      520                      525

ggg aat tgt gac cgg ctg aca ggc agg tgt ttg aag tgt atc cac aac        1749
Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn
        530                      535                      540

aca gcc ggc atc tac tgc gac cag tgc aaa gca ggc tac ttc ggg gac        1797
Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp
545                      550                      555                  560

cca ttg gct ccc aac cca gca gac aag tgt cga gct tgc aac tgt aac        1845
Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn
                565                      570                      575

ccc atg ggc tca gag cct gta gga tgt cga agt gat ggc acc tgt gtt        1893
Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr Cys Val
                580                      585                      590

tgc aag cca gga ttt ggt ggc ccc aac tgt gag cat gga gca ttc agc        1941
Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala Phe Ser
```

```
Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala Phe Ser
        595                 600             605

tgt cca gct tgc tat aat caa gtg aag att cag atg gat cag ttt atg   1989
Cys Pro Ala Cys Tyr Asn Gln Val Lys Ile Gln Met Asp Gln Phe Met
        610                 615             620

cag cag ctt cag aga atg gag gcc ctg att tca aag gct cag ggt ggt   2037
Gln Gln Leu Gln Arg Met Glu Ala Leu Ile Ser Lys Ala Gln Gly Gly
625             630             635             640

gat gga gta gta cct gat aca gag ctg gaa ggc agg atg cag cag gct   2085
Asp Gly Val Val Pro Asp Thr Glu Leu Glu Gly Arg Met Gln Gln Ala
                645             650             655

gag cag gcc ctt cag gac att ctg aga gat gcc cag att tca gaa ggt   2133
Glu Gln Ala Leu Gln Asp Ile Leu Arg Asp Ala Gln Ile Ser Glu Gly
                660             665             670

gct agc aga tcc ctt ggt ctc cag ttg gcc aag gtg agg agc caa gag   2181
Ala Ser Arg Ser Leu Gly Leu Gln Leu Ala Lys Val Arg Ser Gln Glu
        675             680             685

aac agc tac cag agc cgc ctg gat gac ctc aag atg act gtg gaa aga   2229
Asn Ser Tyr Gln Ser Arg Leu Asp Asp Leu Lys Met Thr Val Glu Arg
        690             695             700

gtt cgg gct ctg gga agt cag tac cag aac cga gtt cgg gat act cac   2277
Val Arg Ala Leu Gly Ser Gln Tyr Gln Asn Arg Val Arg Asp Thr His
705             710             715             720

agg ctc atc act cag atg cag ctg agc ctg gca gaa agt gaa gct tcc   2325
Arg Leu Ile Thr Gln Met Gln Leu Ser Leu Ala Glu Ser Glu Ala Ser
                725             730             735

ttg gga aac act aac att cct gcc tca gac cac tac gtg ggg cca aat   2373
Leu Gly Asn Thr Asn Ile Pro Ala Ser Asp His Tyr Val Gly Pro Asn
                740             745             750

ggc ttt aaa agt ctg gct cag gag gcc aca aga tta gca gaa agc cac   2421
Gly Phe Lys Ser Leu Ala Gln Glu Ala Thr Arg Leu Ala Glu Ser His
        755             760             765

gtt gag tca gcc agt aac atg gag caa ctg aca agg gaa act gag gac   2469
Val Glu Ser Ala Ser Asn Met Glu Gln Leu Thr Arg Glu Thr Glu Asp
        770             775             780

tat tcc aaa caa gcc ctc tca ctg gtg cgc aag gcc ctg cat gaa gga   2517
Tyr Ser Lys Gln Ala Leu Ser Leu Val Arg Lys Ala Leu His Glu Gly
785             790             795             800

gtc gga agc gga agc ggt agc ccg gac ggt gct gtg gtg caa ggg ctt   2565
Val Gly Ser Gly Ser Gly Ser Pro Asp Gly Ala Val Val Gln Gly Leu
                805             810             815

gtg gaa aaa ttg gag aaa acc aag tcc ctg gcc cag cag ttg aca agg   2613
Val Glu Lys Leu Glu Lys Thr Lys Ser Leu Ala Gln Gln Leu Thr Arg
            820             825             830

gag gcc act caa gcg gaa att gaa gca gat agg tct tat cag cac agt   2661
Glu Ala Thr Gln Ala Glu Ile Glu Ala Asp Arg Ser Tyr Gln His Ser
            835             840             845
```

```
ctc cgc ctc ctg gat tca gtg tct ccg ctt cag gga gtc agt gat cag    2709
Leu Arg Leu Leu Asp Ser Val Ser Pro Leu Gln Gly Val Ser Asp Gln
    850             855                 860

tcc ttt cag gtg gaa gaa gca aag agg atc aaa caa aaa gcg gat tca    2757
Ser Phe Gln Val Glu Glu Ala Lys Arg Ile Lys Gln Lys Ala Asp Ser
865             870                 875                 880

ctc tca agc ctg gta acc agg cat atg gat gag ttc aag cgt aca caa    2805
Leu Ser Ser Leu Val Thr Arg His Met Asp Glu Phe Lys Arg Thr Gln
                885                 890                 895

aag aat ctg gga aac tgg aaa gaa gaa gca cag cag ctc tta cag aat    2853
Lys Asn Leu Gly Asn Trp Lys Glu Glu Ala Gln Gln Leu Leu Gln Asn
                900                 905                 910

gga aaa agt ggg aga gag aaa tca gat cag ctg ctt tcc cgt gcc aat    2901
Gly Lys Ser Gly Arg Glu Lys Ser Asp Gln Leu Leu Ser Arg Ala Asn
        915                 920                 925

ctt gct aaa agc aga gca caa gaa gca ctg agt atg ggc aat gcc act    2949
Leu Ala Lys Ser Arg Ala Gln Glu Ala Leu Ser Met Gly Asn Ala Thr
    930                 935                 940

ttt tat gaa gtt gag agc atc ctt aaa aac ctc aga gag ttt gac ctg    2997
Phe Tyr Glu Val Glu Ser Ile Leu Lys Asn Leu Arg Glu Phe Asp Leu
945             950                 955                 960

cag gtg gac aac aga aaa gca gaa gct gaa gaa gcc atg aag aga ctc    3045
Gln Val Asp Asn Arg Lys Ala Glu Ala Glu Glu Ala Met Lys Arg Leu
                965                 970                 975

tcc tac atc agc cag aag gtt tca gat gcc agt gac aag acc cag caa    3093
Ser Tyr Ile Ser Gln Lys Val Ser Asp Ala Ser Asp Lys Thr Gln Gln
                980                 985                 990

gca gaa aga gcc ctg ggg agc gct  gct gct gat gca cag  agg gca aag    3141
Ala Glu Arg Ala Leu Gly Ser Ala Ala Ala Asp Ala Gln Arg Ala Lys
        995                 1000                1005

aat ggg  gcc ggg gag gcc ctg  gaa atc tcc agt gag  att gaa cag    3186
Asn Gly Ala Gly Glu Ala Leu  Glu Ile Ser Ser Glu  Ile Glu Gln
    1010                1015                1020

gag att  ggg agt ctg aac ttg  gaa gcc aat gtg aca  gca gat gga    3231
Glu Ile Gly Ser Leu Asn Leu  Glu Ala Asn Val Thr  Ala Asp Gly
    1025                1030                1035

gcc ttg  gcc atg gaa aag gga  ctg gcc tct ctg aag  agt gag atg    3276
Ala Leu Ala Met Glu Lys Gly  Leu Ala Ser Leu Lys  Ser Glu Met
    1040                1045                1050

agg gaa  gtg gaa gga gag ctg  gaa agg aag gag ctg  gag ttt gac    3321
Arg Glu Val Glu Gly Glu Leu  Glu Arg Lys Glu Leu  Glu Phe Asp
    1055                1060                1065

acg aat  atg gat gca gta cag  atg gtg att aca gaa  gcc cag aag    3366
Thr Asn Met Asp Ala Val Gln  Met Val Ile Thr Glu  Ala Gln Lys
    1070                1075                1080

gtt gat  acc aga gcc aag aac  gct ggg gtt aca atc  caa gac aca    3411
Val Asp Thr Arg Ala Lys Asn  Ala Gly Val Thr Ile  Gln Asp Thr
    1085                1090                1095
```

```
ctc aac  aca tta gac ggc ctc  ctg cat ctg atg ggt  atg tga          3453
Leu Asn  Thr Leu Asp Gly Leu  Leu His Leu Met Gly  Met
    1100                  1105                 1110

acccacaacc cacaaccttc cagctccatg ctccagggct ttgctccaga acactcacta   3513

tacctagccc cagcaaaggg gagtctcagc tttccttaag gatatcagta aatgtgcttt   3573

gtttccaggc ccagataact ttcggcaggt tcccttacat ttactggacc ctgttttacc   3633

gttgctaaga tgggtcactg aacacctatt gcacttgggg gtaaaggtct gtgggccaaa   3693

gaacaggtgt atataagcaa cttcacagaa cacgagacag cttgggaatc ctgctaaaga   3753

gtctggcctg gaccctgaga agccagtgga cagtttttaag cagaggaata acatcaccac   3813

tgtatatttc agaaagatca ctagggcagc cgagtggagg aaagcttgaa gaggggggtta   3873

gagagaaggc aggttgagac tacttaagat attgttgaaa taattgaaga gagaaatgac   3933

aggagcctgc tctaaggcag tagaatggtg gctgggaaga tgtgaaggaa gattttccca   3993

gtctgtgaag tcaagaatca cttgccggcc gggtgtggtg gctcacgcct gtaattctag   4053

cactttggga gactgaagcg ggtggatcac ccgaggtcag gagttgaaga ccagcctggc   4113

caacatggtg aaaccctgtc tctactaaaa gtacaaaaat tagctggatg atggtggtgg   4173

gcgcctgtaa ttccagctac tcaggagtct gaggcaggag aatcgcttga acccaggagg   4233

cgaggttaca gtgagccaag attgcaccac tgctcttcca gcctgggaac agagagactg   4293

cctaaaaaaa aaaaaaaaaa aaa                                            4316
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<210> 4
<211> 1111
<212> PRT
<213> Homo sapiens

<400> 4

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Met Pro Ala Leu Trp Leu Gly Cys Cys Leu Cys Phe Ser Leu Leu Leu
1                5                  10                  15


Pro Ala Ala Arg Ala Thr Ser Arg Arg Glu Val Cys Asp Cys Asn Gly
            20                  25                  30


Lys Ser Arg Gln Cys Ile Phe Asp Arg Glu Leu His Arg Gln Thr Gly
            35                  40                  45


Asn Gly Phe Arg Cys Leu Asn Cys Asn Asp Asn Thr Asp Gly Ile His
    50                  55                  60


Cys Glu Lys Cys Lys Asn Gly Phe Tyr Arg His Arg Glu Arg Asp Arg
65                  70                  75                  80
```

Cys Leu Pro Cys Asn Cys Asn Ser Lys Gly Ser Leu Ser Ala Arg Cys
                  85   90   95

Asp Asn Ser Gly Arg Cys Ser Cys Lys Pro Gly Val Thr Gly Ala Arg
            100   105   110

Cys Asp Arg Cys Leu Pro Gly Phe His Met Leu Thr Asp Ala Gly Cys
        115   120   125

Thr Gln Asp Gln Arg Leu Leu Asp Ser Lys Cys Asp Cys Asp Pro Ala
    130   135   140

Gly Ile Ala Gly Pro Cys Asp Ala Gly Arg Cys Val Cys Lys Pro Ala
145   150   155   160

Val Thr Gly Glu Arg Cys Asp Arg Cys Arg Ser Gly Tyr Tyr Asn Leu
            165   170   175

Asp Gly Gly Asn Pro Glu Gly Cys Thr Gln Cys Phe Cys Tyr Gly His
            180   185   190

Ser Ala Ser Cys Arg Ser Ser Ala Glu Tyr Ser Val His Lys Ile Thr
    195   200   205

Ser Thr Phe His Gln Asp Val Asp Gly Trp Lys Ala Val Gln Arg Asn
210   215   220

Gly Ser Pro Ala Lys Leu Gln Trp Ser Gln Arg His Gln Asp Val Phe
225   230   235   240

Ser Ser Ala Gln Arg Leu Asp Pro Val Tyr Phe Val Ala Pro Ala Lys
            245   250   255

Phe Leu Gly Asn Gln Gln Val Ser Tyr Gly Gln Ser Leu Ser Phe Asp
            260   265   270

Tyr Arg Val Asp Arg Gly Gly Arg His Pro Ser Ala His Asp Val Ile
    275   280   285

Leu Glu Gly Ala Gly Leu Arg Ile Thr Ala Pro Leu Met Pro Leu Gly
    290   295   300

Lys Thr Leu Pro Cys Gly Leu Thr Lys Thr Tyr Thr Phe Arg Leu Asn
305   310   315   320

Glu His Pro Ser Asn Asn Trp Ser Pro Gln Leu Ser Tyr Phe Glu Tyr
            325   330   335

Arg Arg Leu Leu Arg Asn Leu Thr Ala Leu Arg Ile Arg Ala Thr Tyr
            340   345   350

Gly Glu Tyr Ser Thr Gly Tyr Ile Asp Asn Val Thr Leu Ile Ser Ala
        355   360   365

Arg Pro Val Ser Gly Ala Pro Ala Pro Trp Val Glu Gln Cys Ile Cys
    370   375   380

Pro Val Gly Tyr Lys Gly Gln Phe Cys Gln Asp Cys Ala Ser Gly Tyr
385   390   395   400

Lys Arg Asp Ser Ala Arg Leu Gly Pro Phe Gly Thr Cys Ile Pro Cys
            405   410   415

Asn Cys Gln Gly Gly Gly Ala Cys Asp Pro Asp Thr Gly Asp Cys Tyr
            420   425   430

Ser Gly Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly
        435   440   445

Phe Tyr Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys
    450   455   460

His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val
465   470   475   480

Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys
            485   490   495

Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg
        500   505   510

Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser
    515   520   525

Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn
530   535   540

Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp
545   550   555   560

Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn
            565   570   575

Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr Cys Val
            580   585   590

Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala Phe Ser
        595   600   605

Cys Pro Ala Cys Tyr Asn Gln Val Lys Ile Gln Met Asp Gln Phe Met
    610   615   620

Gln Gln Leu Gln Arg Met Glu Ala Leu Ile Ser Lys Ala Gln Gly Gly
625   630   635   640

Asp Gly Val Val Pro Asp Thr Glu Leu Glu Gly Arg Met Gln Gln Ala
                645   650   655

Glu Gln Ala Leu Gln Asp Ile Leu Arg Asp Ala Gln Ile Ser Glu Gly
            660   665   670

Ala Ser Arg Ser Leu Gly Leu Gln Leu Ala Lys Val Arg Ser Gln Glu
        675   680   685

Asn Ser Tyr Gln Ser Arg Leu Asp Asp Leu Lys Met Thr Val Glu Arg
    690   695   700

Val Arg Ala Leu Gly Ser Gln Tyr Gln Asn Arg Val Arg Asp Thr His
705   710   715   720

Arg Leu Ile Thr Gln Met Gln Leu Ser Leu Ala Glu Ser Glu Ala Ser
            725   730   735

Leu Gly Asn Thr Asn Ile Pro Ala Ser Asp His Tyr Val Gly Pro Asn
            740   745   750

Gly Phe Lys Ser Leu Ala Gln Glu Ala Thr Arg Leu Ala Glu Ser His
        755   760   765

Val Glu Ser Ala Ser Asn Met Glu Gln Leu Thr Arg Glu Thr Glu Asp
    770   775   780

Tyr Ser Lys Gln Ala Leu Ser Leu Val Arg Lys Ala Leu His Glu Gly
785   790   795   800

Val Gly Ser Gly Ser Gly Ser Pro Asp Gly Ala Val Val Gln Gly Leu
            805   810   815

Val Glu Lys Leu Glu Lys Thr Lys Ser Leu Ala Gln Gln Leu Thr Arg
            820   825   830

Glu Ala Thr Gln Ala Glu Ile Glu Ala Asp Arg Ser Tyr Gln His Ser

```
          835                    840                    845
```

```
Leu Arg Leu Leu Asp Ser Val Ser Pro Leu Gln Gly Val Ser Asp Gln
    850             855             860
```

```
Ser Phe Gln Val Glu Glu Ala Lys Arg Ile Lys Gln Lys Ala Asp Ser
865             870             875             880
```

```
Leu Ser Ser Leu Val Thr Arg His Met Asp Glu Phe Lys Arg Thr Gln
            885             890             895
```

```
Lys Asn Leu Gly Asn Trp Lys Glu Glu Ala Gln Gln Leu Leu Gln Asn
            900             905             910
```

```
Gly Lys Ser Gly Arg Glu Lys Ser Asp Gln Leu Leu Ser Arg Ala Asn
        915             920             925
```

```
Leu Ala Lys Ser Arg Ala Gln Glu Ala Leu Ser Met Gly Asn Ala Thr
    930             935             940
```

```
Phe Tyr Glu Val Glu Ser Ile Leu Lys Asn Leu Arg Glu Phe Asp Leu
945             950             955             960
```

```
Gln Val Asp Asn Arg Lys Ala Glu Ala Glu Glu Ala Met Lys Arg Leu
                965             970             975
```

```
Ser Tyr Ile Ser Gln Lys Val Ser Asp Ala Ser Asp Lys Thr Gln Gln
            980             985             990
```

```
Ala Glu Arg Ala Leu Gly Ser Ala  Ala Ala Asp Ala Gln  Arg Ala Lys
        995             1000            1005
```

```
Asn Gly  Ala Gly Glu Ala Leu  Glu Ile Ser Ser Glu  Ile Glu Gln
    1010            1015            1020
```

```
Glu Ile  Gly Ser Leu Asn Leu  Glu Ala Asn Val Thr  Ala Asp Gly
    1025            1030            1035
```

```
Ala Leu  Ala Met Glu Lys Gly  Leu Ala Ser Leu Lys  Ser Glu Met
    1040            1045            1050
```

```
Arg Glu  Val Glu Gly Glu Leu  Glu Arg Lys Glu Leu  Glu Phe Asp
    1055            1060            1065
```

```
Thr Asn  Met Asp Ala Val Gln  Met Val Ile Thr Glu  Ala Gln Lys
    1070            1075            1080
```

Val Asp Thr Arg Ala Lys Asn Ala Gly Val Thr Ile Gln Asp Thr
    1085   1090   1095

Leu Asn Thr Leu Asp Gly Leu Leu His Leu Met Gly Met
    1100   1105   1110

<210> 5
<211> 530
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Portion of Domain III of laminin gamma 2

<400> 5
aattctgcca ggattgtgct tctggctaca agagagattc agcgagactg gggccttttg   60

gcacctgtat tccttgtaac tgtcaagggg gaggggcctg tgatccagac acaggagatt   120

gttattcagg ggatgagaat cctgacattg agtgtgctga ctgcccaatt ggtttctaca   180

acgatccgca cgaccccgc agctgcaagc catgtccctg tcataacggg ttcagctgct   240

cagtgattcc ggagacggag gaggtggtgt gcaataactg ccctcccggg gtcaccggtg   300

cccgctgtga gctctgtgct gatggctact ttggggaccc ctttggtgaa catggcccag   360

tgaggccttg tcagccctgt caatgcaaca gcaatgtgga ccccagtgcc tctgggaatt   420

gtgaccggct gacaggcagg tgtttgaagt gtatccacaa cacagccggc atctactgcg   480

accagtgcaa agcaggctac ttcggggacc cattggctcc caacccagca   530

<210> 6
<211> 177
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Portion of Domain III of laminin gamma 2.

<400> 6

Gln Phe Cys Gln Asp Cys Ala Ser Gly Tyr Lys Arg Asp Ser Ala Arg
1   5   10   15

Leu Gly Pro Phe Gly Thr Cys Ile Pro Cys Asn Cys Gln Gly Gly Gly
            20   25   30

Ala Cys Asp Pro Asp Thr Gly Asp Cys Tyr Ser Gly Asp Glu Asn Pro
        35   40   45

Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr Asn Asp Pro His

50   55   60

Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn·Gly Phe Ser Cys
65   70   75   80

Ser Val Met Pro Glu Thr Glu Glu Val Val Cys Asn Asn Cys Pro Pro
                85   90   95

Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp Gly Tyr Phe Gly
            100   105   110

Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys Gln Pro Cys Gln
        115   120   125

Cys Asn Asn Asn Val Asp Pro Ser Ala Ser Gly Asn Cys Asp Arg Leu
    130   135   140

Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala Gly Ile Tyr Cys
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Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp Pro Leu Ala Pro Asn Pro
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tgtgctgact gcccaattgg tttctacaac gatccgcacg accccgcag ctgcaagcca   240

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aataactgcc ctcccgggt caccggtgcc cgctgtgagc tctgtgctga tggctacttt   360

ggggacccct ttggtgaaca tggcccagtg aggccttgtc agccctgtca atgcaacagc   420

aatgtggacc ccagtgcctc tgggaattgt gaccggctga caggcaggtg tttgaagtgt   480

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ttggctccca acccagcaga caagtgtcga gcttgcaact gtaaccccat gggctcagag   600

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Ile Pro Cys Asn Cys Gln Gly Gly Ala Cys Asp Pro Asp Thr Gly
            35   40   45

Asp Cys Tyr Ser Gly Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys
        50   55   60

Pro Ile Gly Phe Tyr Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro
65   70   75   80

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Met Pro Glu Thr Glu
                85   90   95

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
                100   105   110

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
            115   120   125

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Asn Asn Val Asp Pro
        130   135   140

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
145   150   155   160

Ile His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr
                165   170   175

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Phe Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys
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Asn Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly
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Thr Cys Val Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly
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Ala Phe Ser
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Gly Cys Arg Ser Asp Gly Thr Cys Val Cys Lys Pro Gly Phe Gly Gly
            20              25              30


Pro Asn Cys Glu His Gly Ala Phe Ser
        35              40
```

(51) International Patent Classification[7]: A61K 39/395, C07K 16/18, G01N 33/574

(21) International Application Number:
PCT/EP2003/012012

(22) International Filing Date: 29 October 2003 (29.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/422,009   29 October 2002 (29.10.2002)   US

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(74) Agent: VOSSIUS, Volker; Geibelstrasse 6, 81679 Munich (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
3 June 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF ANTIBODIES TO THE GAMMA 2 CHAIN OF LALLMININ 5 TO INHIBIT TUMOR GROWTH AND METASTASIS

(57) Abstract: The present invention provides a methods and compositions for inhibiting tumor growth and/or metastasis involving the administering to a subject with a laminin 5-secreting tumor of an amount effective to inhibit tumor growth and/or metastasis of an antibody that binds to one or more epitopes of the laminin 5 γ2 chain.

INTE... ATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   A61K39/395   C07K16/18   G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   A61K   C07K   G01N   C12Q   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/052307 A1 (KALLUNKI PEKKA ET AL) 2 May 2002 (2002-05-02) see interalia (0091) and (0106) ---	1-13
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2004	2 6 MAR 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	MICAEL OWALD /EÖ

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page 1 of 2

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Application No

PCT/EP 03/12012

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